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(54) PROCESS FOR PRODUCING TRANSFORMED CELL

(57) A process for producing transformed cells by introducing foreign genes into target cells through piercing, which comprises the step of culturing the target cells having the foreign genes injected thereinto in the presence of a cell adhesion-active substance; and a kit for producing transformed cells suitable for use in the above method and containing as the essential ingredients the cells to be transformed with foreing genes by this method and a cell adhesion-active substance.

Description

TECHNICAL FIELD

The present invention relates to a method for production of transfected cells, more particularly, a method which makes possible to effectively transfer a foreign gene into target cells in the field such as cell technology, genetic engineering, developmental engineering and the like.

BACKGROUND ART

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As a method for transferring a foreign gene into target cells, there are known a calcium phosphate method, a DEAE-dextran method, a liposome method, an electroporation method, a microinjection method, a particle gun method and the like. All of these methods have advantages and disadvantages in respect of manipulation procedures, efficacy, damage on cells and the like. Among these methods, a perforation method such as an electroporation method, a microinjection method, a particle gun method and the like can easily handle cells without using special reagents and have good transfer efficacy. However, damage of cells by perforation can not be avoided.

The object of the present invention is to provide a method for improving the transfer efficacy when a foreign gene is transferred into target cells by a perforation method to produce transfected cells.

SUMMARY OF THE INVENTION

The first aspect of the present invention relates to a method for production of transfected cells and is characterized in that said aspect includes a step of, after injection of a foreign gene into target cells using a perforation method, culturing the cells in the presence of a cell-adhering active substance, in a method for production of a transfected cell using a perforation method.

The second aspect of the present invention relates to gene-transferred cells which are produced by the method of the present invention.

The third aspect of the present invention relates to a kit for production of transfected cells, which is used for a method for production of transfected cells according to the first aspect of the present invention and is characterized in that said aspect contains a cell-adhering active substance.

DETAILED DESCRIPTION OF THE INVENTION

The method of the present invention is characterized in that, after a foreign gene is transferred into target cells using a perforation method, the cell is cultured in the presence of a substance having the cell adhesive activity.

As used herein, the perforation method means a method for injection of a gene by perforating a cell wall, including an electroporation method, a microinjection method, a particle gun method and the like. The electroporation method is as described in, for example, Tanpakushitsu, Kakusan, Koso, volume 31, page 1591-1603 (1986). The microinjection method is as described in, for example, Cell, volume 22, page 479-488 (1980). The particle gun method is as described in, for example, Technique, volume 3, page 3-16 (1991). These methods include the known methods used for transferring a gene into cells.

For cells used in these perforation methods, for example, animal cells may be prepared according to a known method ["Shin-Seikagaku Jikkenkoza 18, Saibobaiyogijyutsu", 1st edition (1990), edited by Nippon Seikagakugakkai, published by Tokyo Kagakudojin] or cultured animal cells may be used.

As used herein, a cell-adhering active substance refers to a substance having the cell-adhering activity, that is, the activity to make target cells adhere to a cell, or to an extracellular matrix which is a substance filling a space between cells in the tissue, or to a material such as plastic, glass and the like. In the present invention, any substances having the activity can be used as long as they give no adverse effects on transfection of target cells. Such the activity is to fix cells, for example, to a culture wear covered with a cell-adhering active substance while maintaining the cell in its form, or in the spreaded form, that is, in the changed form after the cell has been spreaded in one or more directions.

Attachment between the cell-adhering active substance and the target cell can be assayed using a conventional method. The method includes, for example, a method described in Nature, 352: 438-441 (1991). Briefly, the cell-adhering active substance covers a plastic dish and a population of cells to be assayed is put into medium, allowing to stand for 30 minutes to 2 hours. After this incubation period, non-adhered cells are recovered, counted and assayed for viability. Cells adhered to the cell-adhering active substance are recovered using trypsin or a cell dissociation buffer (for example, Gibco), counted and tested for viability. Then, a proportion of adhered cells is calculated and compared with standard or standard control such as a plastic dish covered with bovine serum albumin (BSA). A combination of cell-adhering active substance/cell can be determined by substantial adhesion of the target cell with the cell-adhering active substance assayed. In addition, the cell-spreading activity can be determined by observing under a microscope a

change in the form before adhered cells are dissociated using trypsin or a cell dissociation buffer, in the above procedures

Examples of the cell-adhering active substance include, for example, a cell-adhering active polypeptide or a functional equivalent thereof and a cell-adhesive synthetic polymer.

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Examples of the polypeptide, used in the present invention, having the cell-adhering activity include a cell-adhering active polypeptide such as invasin, polylysine and the like other than that derived from extracellular matrix, for example, a polypeptide showing the cell-spreading activity described in JP-A 2-311498, for example, components of an extracellular matrix such as fibronectin, laminin, collagen, vitronectin, osteopontin, thrombospondin, tenasin and the like. The extracellular matrix components can be prepared from a natural or cultured source by the known method [International Journal of Cancer, volume 20, page 1-5 (1977); Journal of Biological Chemistry, volume 254, page 9933-9937, (1979); "Zoku-Seikagaku Jikkenkoza, volume 6, Saibokokkaku no Kozo to Kino (Structure and Function of Cell Skeleton) (last volume), (1st edition) (1986) edited by Nippon Seikagakugakkai, published by Tokyo Kagakudojin; Cell Structure and Function, volume 13, page 281-292 (1988); Journal of Biological Chemistry, volume 264, page 18202-18208 (1989); and Journal of Biological Chemistry, volume 260, page 12240-12245 (1985)]. The cell-adhering active polypeptide may be substantially purified extracellular matrices exhibiting the cell-adhering activity, substantially purified extracellular matrix fragments or a mixture thereof. More particularly, proteins and polypeptides having the cell-adhering activity or the cell-spreading activity, or a functional equivalent thereof may be used.

As these cell-adhering active polypeptides, substantially purified natural polypeptides, polypeptides from enzymological or chemical degradation of the natural polypeptides, or the similar polypeptides made by genetic engineering may be used. Further, materials obtained by altering these polypeptides without impairing the function, that is, the cell-adhering activity or the cell-spreading activity may be used. In the present invention, even when the amino acid sequence of a polypeptide from natural origin has deletion, substitution, addition and/or insertion of an amino acid, as long as the polypeptide has the desired cell-adhering activity or the cell-spreading activity, it is referred to as a functional equivalent of a polypeptide having the natural amino acid sequence. That is, it is known that naturally occurring proteins include proteins of which amino acid sequences have mutation such as deletion, insertion, addition, substitution and the like of an amino acid due to modification reaction in the living body after production or during purification, in addition to proteins having a change in the amino acid sequence due to polymorphism or mutation of genes encoding those naturally occurring proteins and that, regardless of these, there are proteins exhibiting the physiological and biological activity substantially equivalent to that of proteins having no mutation. Like this, even when there is a structural difference between polypeptides, as long as they share the common main functions, they are called polypeptides having the functionally equivalent activity.

This is also true where the above mutations are artificially introduced into the amino acid sequence of proteins. In this case, more variety of mutants may be made. As long as these mutants exhibit the physiological activity substantially equivalent to that of proteins having no mutation, they are interpreted to be a polypeptide having the functionally equivalent activity.

For example, in many cases, a methionine residue present at a N-terminal of a protein expressed in Escherichia coli is said to be removed by an action of methionine aminopeptidase, thus, generating both proteins having a methionine residue or those having no methionine residue depending upon the kind of proteins. However, whether or not a protein has a methionine residue dose not affect on the protein activity in many cases. In addition, it is known that a polypeptide where a certain cysteine residue is substituted with a serine residue in the amino acid sequence of human interleukin-2 (IL-2) retains the interleukin-2 activity [Science, volume 224, page 1431 (1984)].

Further, upon production of proteins by genetic engineering, it is frequently conducted that the proteins are expressed as a fused protein. For example, in order to increase an amount of an expressed protein of interest, it is conducted that the protein is expressed by adding a N-terminal peptide chain derived from other protein to a N-terminal of the protein of interest, or adding a suitable peptide chain to a N-terminal or a C-terminal of the protein of interest to facilitate purification of the protein of interest by using a carrier having the affinity to the added peptide chain.

In this respect, the related biotechnological techniques have progressed and, as the result, deletion, substitution, addition or other modification of an amino acid in a functional area of a subject can be routinely carried out. Then, the resulting amino acid sequence may be routinely screened for the desired cell-adhering activity or the cell-spreading activity according to the above method.

Polypeptides having the cell-adhering activity may be an artificial polypeptide containing, in the molecule, the amino acid sequence necessary for the cell-adhering activity, for example, the amino acid sequence may be selected from the amino acid sequence represented by SEQ ID: No. 1 (RGDS), the amino acid sequence represented by SEQ ID: No. 6 (central sequence of laminin, YIGSR). These polypeptides can be prepared in a large amount by a genetic engineering method or chemical synthesis method and may be used as a purified polypeptide.

Examples of the artificial polypeptide having, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 include a polypeptide represented by SEQ ID: NO. 7 described in JP-A 1-180900. The polypeptide can be prepared using Escherichia coli HB101/pTF1409 (FERM BP-1939) according to a method described in JP-A 1-180900. In

addition polypeptides represented by respective sequence ID numbers in the sequence list shown in Table 1 below can be prepared according to a genetic engineering method described in each specification.

In addition, a plasmid HB101/pCHV90 contained in Escherichia coli HB101/pCHV90 in Table 1 can be prepared using Escherichia coli HB101/pHD101 (FERM BP-2264) and Escherichia coli JM109/pTF7021 (FERM BP-1941) according to a method described in JP-A 5-271291.

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Table 1

| Laid Open publication | SEQ ID: No. | Living bacterium (Escherichia coli) | Accession No. |
|-----------------------|-------------|-------------------------------------|---------------|
| JP-A 1-206998 | 8 | JM109/pTF7021 | FERM BP-1941 |
| JP-A 1-261398 | 9 | HB101/pTF1801 | FERM P-9948 |
| JP-A 2-97397 | 3 | JM109/pTF7221 | FERM BP-1915 |
| JP-A 2-152990 | 10 | JM109/pTFB800 | FERM BP-2126 |
| JP-A 2-311498 | 11 | HB101/pCH101 | FERM BP-2799 |
| JP-A 3-59000 | 12 | JM109/pCF406 | FERM P-10837 |
| JP-A 3-232898 | 13 | HB101/pCE102 | FERM P-11226 |
| JP-A 4-54199 | 14 | JM109/pTF7520 +VN-IN.TAA | FERM P-11526 |
| | 15 | JM109/pTF7520 +Col ^{X1} | FERM P-11527 |
| JP-A 5-271291 | 16 | HB101/pCHV179 | FERM P-12183 |
| | 17 | HB101/pCHV90 | - |
| | 18 | HB101/pCHV89 | FERM P-182 |
| JP-A 5-97698 | 19 | JM109/pTF7520CoIV | FERM BP-5277 |
| JP-A 5-178897 | 20 | JM109/pYMH-CF • A | FERM BP-5278 |

Alternatively, artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 can be chemically synthesized. For example, PolyRGDS described in JP-A 3-173828 can be synthesized and used.

Examples of artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 include a polypeptide represented by SEQ ID: No. 4 described in JP-A 2-311498 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pHD102 (FERM P-10721) according to a method described in JP-A 2-311498. In addition, a polypeptide represented by SEQ ID: No. 2 may be chemically synthesized according to a method described in JP-A 3-284700.

Further, examples of artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 and the amino acid sequence represented by SEQ ID: No. 3 include a polypeptide represented by SEQ ID: No. 21 described in JP-A 2-311498 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pCH102 (FERM BP-2800) according to a method described in JP-A 2-311498. In addition, a polypeptide represented by SEQ ID: No. 5 described in JP-A 3-284700 is a polypeptide containing, in the molecule, the amino acid sequences of SEQ ID: No. 1 and 2 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pCS25 (FERM P-11339) according to a method described in JP-A 3-284700.

As described above, examples of the polypeptides used in the present invention are cell-adhering active polypeptides containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2. As the polypeptide, a polypeptide obtained by covalently binding a polypeptide derived from a cell adhesion domain of human fibronectin ["Fibronectin", page 47-121 (1989), edited by Mosher, D.F., published by Academic Press] with a CS1 polypeptide derived from the same (ibid), a polypeptide derived from a heparin binding domain (ibid) containing a CS1 polypeptide, or a polypeptide derived from cell adhesion can be used, and they can be made by genetic engineering, respectively. For example, respective necessary regions are taken out from a vector containing a DNA encoding a cell adhesion domain-derived polypeptide, a vector containing a DNA encoding a CS1 polypeptide, and a vector containing a DNA encoding a heparin binding domain-derived peptide containing a CS1 polypeptide, respectively, and they can be used alone or in combination thereof to make a vector expressing a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2.

When a polypeptide where a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 are covalently bound is made, a covalent bonding between polypeptides may be a direct bonding or an indirect bonding, for example, an indirect bonding via a spacer. A spacer is an insertion sequence for adjusting an intermolecular distance in each region. As the spacer, an arbitral peptide chain can be used, for example, a sequence upstream of a CS1 region in fibronectin molecule. The spacer sequence can be easily introduced therein by genetic engineering.

The cell-adhesive synthetic polymers include the known poly-N-p-vinylbenzyl-D-lactoneamide (PVLA).

In the present invention, the target cell include, but being not limited to, hematopoiesis stem cell, peripheral blood stem cell, umbilical blood cell, ES cell, lymphocyte, cancer cell and the like.

Examples of the foreign gene include, but being not limited to, nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes). In the present invention, the foreign gene may be inserted into a vector.

Examples of the vector are retrovirus vector, adenovirus vector, vacciniavirus vector, herpesvirus vector and the like.

According to the present invention, a target cell into which a foreign gene has been transferred by a perforation method according to a conventional method can be cultured in the presence of a cell-adhering active substance to effectively obtain transfected cells with a transferred gene. A cell culture method may be selected from the known methods depending upon a cell used. For example, when cell culturing is performed in the presence of a cell-adhering active polypeptide, 250 to 2000 μ g/ml of the cell-adhering active polypeptide may be used in a culture medium to culture it according to a conventional method.

Particularly, culturing is preferably carried out using a culture wear covered with a cell-adhering active substance. The culture wear refers to any wear normally used for cell culture, for example, a culture dish, a culture wear using a microcarrier, and a culture wear using fibrous hollow fibers. The culture wear may be covered with the substance by coating or spraying. For example, the culture wear may be easily covered with the cell-adhering active substance. The culture wear may be easily covered with the polypeptide by dissolving it in a suitable solution such as a phosphate buffered saline (PBS), adding the solution to the culture wear and allowing to stand for a suitable period of time. An amount of the polypeptide with which the culture wear is covered may be selected from a range of 50 to 1000 pmol/cm², suitably 150 to 600 pmol/cm².

Transfected cells which have been cultured in the presence of the cell-adhering active substance can be obtained from a culture according to a conventional method. Thus, transfected cells can be produced effectively.

The resulting transfected cells are useful for production of useful substances by cells using gene recombination techniques, exploitation of disease models, gene therapy and the like. Thus, transfected cells can be effectively produced according to the present invention.

In addition, the present invention can be simply carried out by using a kit containing a cell-adhering active substance. The cell-adhering active substance to be contained in the kit may be in a form of solutions or lyophilized powders. The kit may contain a buffer for dissolving or diluting the cell-adhering active substance, a cell culture medium, a cell culture wear and the like. For example, a transfected cell can be simply produced by preparing a kit combining polypeptides, PBS for diluting the polypeptide, a cell culture wear and the like which are used for the method of the present invention. A reagent contained in the kit may be liquid or lyophilized.

A perforation method in the present invention can be used by appropriately selecting from an electroporation method, a microinjection method, a particle gun method and the like depending upon the purpose.

The present invention is illustrated by Examples below but is not limited to them.

45 Example 1

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1. Coating of cell-adhering active polypeptide on culture dish

A polypeptide represented by SEQ ID: No. 3 (hereinafter referred to as "C274"), a polypeptide represented by SEQ ID: No. 4 (hereinafter referred to as "H296") and a polypeptide represented by SEQ ID: No. 5 (hereinafter referred to as "C • CS1") were dissolved in a phosphate buffered saline (PBS) to each 1 μM, respectively, which were steriled using a 0.22 μm filter (Millex-GV, Millipore).

Each 1 ml/well of these solutions was added to a 24-well polystyrene culture dish (manufactured by Corning), respectively, to coat the dish at 4 °C overnight. These dishes were rinsed with a 500 μ l/well of a Dulbecco's modified minimum basal medium containing no bovine fetal serum prior to addition of a transformed cell described below.

2. Transfection of cells

Two culture dishes (diameter: 100 mm) of human epidermoid cancer cell A-431 which had been cultured in a Dul-

becco's modified minimum basal medium containing 10% bovine fetal serum were rinsed with 10 ml of a Dulbecco's modified minimum basal medium containing no bovine fatal serum, respectively, and 3 ml of PBS containing 0.25% bovine trypsin and 0.02% EDTA was added thereto to detach cells from the culture dish. To these was added 7 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were suspended in 10 ml of a Dulbecco's modified minimum basal medium containing bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were combined, suspended in 10 ml of PBS, a 3/10 aliquot of the suspension was taken and divided into two equal aliquots, which were centrifuged at 800 rpm for 3 minutes to collect cells, respectively. The resulting cells were suspended again in 10 ml of PBS, followed by centrifugation at 800 rpm for 3 minutes to collect two batches of cells. One batch of the resulting cells were suspended in 1 ml of PBS containing 15 μg of pCAT-control vector (Promega) which had been aseptically prepared, and placed in an electroporation cuvette for Gene Pulser (BioRad), which were allowed to stand in ice for 10 minutes. The other batch of the resulting cells were suspended in 1 ml of PBS, and placed in an electroporation cuvette for Gene Pulser (BioRad), which were allowed to stand in ice for 10 minutes. Each batch of cells were allowed to stand in ice for 10 minutes, and voltage was applied thereto at 250V and 960 µF. After application, the cells were allowed to stand in a cuvette in ice for 10 minutes. Thereafter, the cells were recovered into 15 ml of a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum, 1 ml/well of which were added to a 24-well polystyrene culture dish covered with the above polypeptide. These cells were cultured at 37 °C in the presence of 5% CO₂ gas overnight, the medium was removed by aspiration, and 1 ml/well of a fresh Dulbecco's modified minimum basal medium containing 10% bovine fetal serum was added thereto, followed by culturing at 37 °C in the presence of 5% CO2 gas overnight.

3. Determination of transfection efficacy (efficacy of gene transfer)

The cultured cells were rinsed three times with 1.25 ml of PBS per well, a lysed cell solution was prepared, and detection of expressed CAT was carried out using CAT-ELISA kit (manufactured by Boehringer Mannheim) according to a method for using the present kit. Since the present kit used a horseradish peroxidase-labelled secondary antibody and ABTS as a substrate, a ratio of 405nm/490nm was determined. An value obtained by subtracting a blank value from a value for each group in a case of addition of pCAT-control vector using as a blank a group in a case of no addition of pCAT-control vector upon electroporation was adopted as an amount of expressed CAT.

The results thereof are shown in Fig. 1. That is, Fig. 1 is a view showing efficacy of gene transfer into a cell in each polypeptide-treatment group, where the ordinate shows non-treated group and each polypeptide-treatment group and the abscissa shows gene transfer efficacy expressed as a ratio of absorbance at 405 nm relative to that at 490 nm.

As shown in Fig. 1, an amount of expressed CAT in the culture dish in the C274, H296 or C • CS1-treatment group is higher as compared with that in a non-treatment group, demonstrating that efficacy of transfer of pCAT-control vector into a cell is higher.

Example 2

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1. Coating of cell-adhering active polypeptide on culture dish

A polypeptide represented by SEQ ID: No. 3 (hereinafter referred to as "C274"), a polypeptide represented by SEQ ID: No. 4 (hereinafter referred to as "H296") and a polypeptide represented by SEQ ID: No. 5 (hereinafter referred to as "C \cdot CS1") were dissolved in a phosphate buffered saline (PBS) to each 1 μ M, respectively, which were steriled using a 0.22 μ m filter (Millex-GV, Millipore). 1 ml/well of these solutions were added to a 24-well polystyrene culture dish (manufactured by Corning) to coat the dish at 4 °C overnight, respectively. These dishes were rinsed with 500 μ l/well of a Dulbecco's modified minimum basal medium containing no bovine fetal serum prior to addition of a transformed cell described below.

2. Transfection of cell

Two culture dishes (diameter: 100 mm) of African green monkey kidney cell COS-7 which had been cultured in a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum were rinsed with 10 ml of a Dulbecco's modified minimum basal medium containing no bovine fatal serum, respectively, and 3 ml of PBS containing 0.25% bovine trypsin and 0.02% EDTA was added thereto to detach cells from the culture dish. To these was added 7 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, respectively, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were suspended in 10 ml of a Dulbecco's modified minimum basal medium containing bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were combined, suspended in 12 ml of PBS, a 5/6 aliquot of the suspension was taken and divided into two equal aliquots, which were centrifuged at 800 rpm for 3 minutes to collect cells, respectively. The resulting cells

were suspended in 6 ml of PBS, followed by centrifugation at 800 rpm for 3 minutes to collect two batches of cells. One batch of the resulting cells were suspended in 1 ml of PBS containing 15 μ g of pCAT-control vector (Promega) which had been aseptically prepared, and placed in an electroporation cuvette for Gene Pulser (BioRad), which was allowed to stand in ice for 10 minutes. The other batch of the resulting cells were suspended in 1 ml of PBS, and placed in an electroporation cuvette for Gene Pulser (BioRad), which was allowed to stand in ice for 10 minutes. Each batch of cells were allowed to stand in ice for 10 minutes, and voltage was applied thereto at 250V and 960 μ F. After application, the cells were allowed to stand in a cuvette in ice for 10 minutes. Thereafter, the cells were recovered into 15 ml of a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum, 1 ml/well of the cells were added to a 24-well polystyrene culture dish covered with the above polypeptide. These cells were cultured at 37 °C in the presence of 5% CO₂ gas overnight, the medium was removed by aspiration, and 1 ml/well of a fresh Dulbecco's modified minimum basal medium containing 10% bovine fetal serum was added, followed by culturing at 37 °C in the presence of 5% CO₂ gas overnight.

3. Determination of transfection efficacy (efficacy of gene transfer)

The cultured cells were rinsed three times with 1.25 ml of PBS per well, a lysed cell solution was prepared, and detection of expressed CAT was carried out using CAT-ELISA kit (manufactured by Boehringer Mannheim) according to a method for using the present kit. Since the present kit used a horseradish peroxidase-labelled secondary antibody and ABTS as a substrate, a ratio of 405nm/490nm was determined. An value obtained by subtracting a blank value from a value for each group in a case of addition of pCAT-control vector using as a blank a group in a case of no addition of pCAT-control vector upon electroporation was adopted as an amount of expressed CAT. The results thereof are shown in Fig. 2. That is, Fig. 2 is a view showing efficacy of gene transfer into a cell in each polypeptide-treatment group, where the ordinate shows non-treated group and each polypeptide-treatment group and the abscissa shows gene transfer efficacy expressed as a ratio of absorbance at 405 nm relative to that at 490 nm.

As shown in Fig. 2, an amount of expressed CAT in the culture dish in the above C274, H296 or C • CS1-treatment group is higher as compared with that in a non-treatment group, demonstrating that efficacy of transfer of pCAT-control vector into a cell is higher.

Example 3

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Preparation of kit

A kit for production of gene-transfered cells was made from C274, H296, C \cdot CS1, PBS and a culturing dish as shown in Table 2 below. Reagents A, B and C were prepared so that the above polypeptides were adjusted with PBS to indicated concentrations shown in the Table. Other components were used which are described in Example 1. In addition, all of reagents A, B and C and a diluent for reagents were aseptically prepared by pre-filtering with a 0.22 μ m sterile filter.

Table 2

| Kit for production of transfed | cted cell |
|----------------------------------|-----------|
| Reagent A • • • 100 μM C274 | 150 µl |
| Reagent B • • • 100 μM H296 | 150 µl |
| Reagent C · · · 100 μM C · CS1 | 150 µl |
| Diluent for reagents • • • PBS | 45 ml |
| 24-well polystyrene culture dish | 3 |

As described above, the present invention can overcome the problems of the previous methods for gene transfer into cells and provide a method, for production of transfected cells, having improved efficacy of gene transfer into target cells. The present invention can also provide a kit, for production of transfected cells, which are used for the method.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a graph showing the effect of cell-adhering active polypeptide treatment on gene transfer efficacy in transfer of pCAT-control vector into human epidermoid cancer cell A-431.

Fig. 2 is a graph showing the effect of cell-adhering active polypeptide treatment on gene transfer efficacy in transfer of pCAT-control vector into African green monkey kidney cell COS-7.

Sequence Listing

| 5 | (1) GENERAL INFORMATION: |
|----|---|
| 10 | (i) APPLICANT: (A) NAME: Takara Shuzo Co., Ltd. (B) STREET: 609, Takenaka-cho, Fushimi-ku (C) CITY: Kyoto-shi, Kyoto (E) COUNTRY: Japan (F) ZIP: 612 |
| | (ii) TITLE OF INVENTION: Method for production of transfected cells |
| 15 | (iii) NUMBER OF SEQUENCES: 21 |
| | (iv) COMPUTER READABLE FORM: |
| 20 | (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb (B) COMPUTER: IBM PS/2 Model 50Z or 55SX (C) OPERATING SYSTEM: MS-DOS (Version 5.0) (D) SOFTWARE: Microsoft Word |
| 25 | (v) CURRENT APPLICATION DATA: (A) APPLICATION NUMBER: EP 95 93 8599.8 (B) FILING DATE: |
| 30 | (vi) PRIOR APPLICATION DATA:(A) APPLICATION NUMBER: PCT/JP95/02425(B) FILING DATE: 29. November 1995 |
| 35 | (2) INFORMATION FOR SEQ ID NO: 1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 4 (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear |
| 40 | (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1: Arg Gly Asp Ser 1 |
| 45 | (2) INFORMATION FOR SEQ ID NO:2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 25 (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear |
| 50 | (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2: |
| | Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu His 5 10 15 Gly Pro Glu Ile Leu Asp Val Pro Ser Thr |
| 55 | 20 25 |
| | |

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(2) INFORMATION FOR SEQ ID NO: 3:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 274
              (B) TYPE: amino acid
5
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:
10
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
                                                    10
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                               35
                                                    40
15
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                               50
                                                    55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                                                    70
                               65
              His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                                                    85
20
              Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                               95
                                                   100
              Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                                                   115
                              110
              Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
25
                              125
                                                   130
                                                                       135
              Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                              140
                                                  145
                                                                       150
              Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                              155
                                                   160
              Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
30
                              170
                                                   175
              Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                              185
                                                   190
              Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                              200
                                                   205
              Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
35
                              215
                                                   220
                                                                       225
              Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                              230
                                                   235
              Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                              245
                                                   250
40
              Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                                                  265
                                                                       270
              Thr Glu Ile Asp
              (2) INFORMATION FOR SEQ ID NO: 4:
              (i) SEQUENCE CHARACTERISTICS:
45
              (A) LENGTH: 296
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
50
              Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr Pro
                                                    10
```

9

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Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu Thr
                               20
              Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met
                                                    40
5
              Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser
                                                    55
                               50
              Gly Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu
                                                    70
              Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr
                               80
                                                    85
10
              Leu Glu Asn Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala
                               95
                                                   100
              Thr Glu Thr Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr
                                                                       120
                              110
                                                   115
              Ile Thr Gly Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr
                                                  130
                              125
              Pro Ile Gln Arg Thr Ile Lys Pro Asp Val Arg Ser Tyr Thr Ile
                              140
                                                   145
              Thr Gly Leu Gln Pro Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr
                                                   160
              Leu Asn Asp Asn Ala Arg Ser Ser Pro Val Val Ile Asp Ala Ser
20
                              170
                                                   175
              Thr Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr
                              185
                                                   190
                                                                       195
              Pro Asn Ser Leu Leu Val Ser Trp Gln Pro Pro Arg Ala Arg Ile
                              200
                                                   205
25
              Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly Ser Pro Pro Arg
                              215
                                                   220
              Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile
                              230
                                                   235
              Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala
                               245
                                                   250
                                                                       255
              Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys
                              260
                                                   265
              Thr Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu
                              275
                                                   280
              His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
                              290
                                                   295
35
              (2) INFORMATION FOR SEQ ID NO: 5:
               (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 302
              (B) TYPE: amino acid
40
               (C) STRANDEDNESS: single
               (D) TOPOLOGY: linear
               (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
45
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                                                    25
                               20
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                               35
                                                    40
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
50
                                                    55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                                65
```

| | His | Glu | Ser | Thr | Pro 80 | Leu | Arg | Gly | Arg | Gln 85 | Lys | Thr | Gly | Leu | Asp 90 |
|----|----------|---------------|-------|------|------------|-----|-----|-------|-------|------------|-----|-----|-----|-----|------------|
| | Ser | Pro | Thr | Gly | Ile 95 | Asp | Phe | Ser | Asp | Ile 100 | Thr | Ala | Asn | Ser | Phe 105 |
| 5 | Thr | Val | His | Trp | | Ala | Pro | Arg | Ala | | Ile | Thr | Gly | Tyr | Arg 120 |
| | Ile | Arg | His | His | | Glu | His | Phe | ser | _ | Arg | Pro | Arg | Glu | |
| 10 | Arg | Val | Pro | His | | Arg | Asn | Ser | Ile | | Leu | Thr | Asn | Leu | |
| | Pro | Gly | Thr | Glu | | Val | Val | Ser | Ile | | Ala | Leu | Asn | Gly | |
| | Glu | Glu | Ser | Pro | Leu 170 | Leu | Ile | Gly | Gln | | Ser | Thr | Val | Ser | Asp 180 |
| 15 | Val | Pro | Arg | Asp | | Glu | Val | Val | Ala | | Thr | Pro | Thr | Ser | |
| | Leu | Ile | Ser | Trp | | Ala | Pro | Ala | Val | | Val | Arg | Tyr | Tyr | |
| | Ile | Thr | Tyr | Gly | | Thr | Gly | Gly | Asn | | Pro | Val | Gln | Glu | |
| 20 | Thr | Val | Pro | Gly | | Lys | Ser | Thr | Ala | | Ile | Ser | Gly | Leu | |
| | Pro | Gly | Val | Asp | Tyr 245 | Thr | Ile | Thr | Val | Tyr 250 | Ala | Val | Thr | Gly | |
| | Gly | Asp | ser | Pro | | Ser | Ser | Lys | Pro | Ile 265 | Ser | Ile | Asn | Tyr | Arg 270 |
| 25 | Thr | Glu | Ile | Asp | | Pro | Ser | Asp | Glu | | Pro | Gln | Leu | Val | |
| | Leu | Pro | His | Pro | Asn 290 | Leu | His | Gly | Pro | | Ile | Leu | Asp | Val | |
| | Se | r Thi | r | | | | | | | | | | | | |
| 30 | | INFO | | | | | | | 6: | | | | | | |
| | (A) | LENG TYP | GTH: | 5 | | | | | | | | | | | |
| 35 | | STR | | | | _ | е | | | | | | | | |
| | |) MOI) SE | | | | | | SEQ : | ID NO | o: 6 | : | | | | |
| | | Ile | Gly | Ser | | | | | | | | | | | |
| 40 | 1 | | | | 5 | | | | _ | | | | | | |
| | | INFO SEQ | | | | _ | | | 7: | | | | | | |
| | | LENO TYP | | | aci | Ŀ | | | | | | | | | |
| 45 | | STR | | | | _ | е | | | | | | | | |
| | (ii |) MO | LECU: | LE T | YPE: | pep | | SEQ : | ID N | 0: 7 | : | | | | |
| | _ | | Pro | Pro | Pro | Thr | Asp | Leu | Arg | | Thr | Asn | Ile | Gly | Pro |
| 50 | 1 Asp | | Met | Arg | | Thr | Trp | Ala | Pro | | Pro | Ser | Ile | Asp | 15 Leu |
| | Thr | Asn | Phe | Leu | 20 Val | Arg | Tyr | Ser | Pro | 25 Val | Lys | Asn | Glu | Glu | 30 Asp |
| | | | | | | | | | | | | | | | |

| | | | | | 35 | | | | | 40 | | | | | 45 |
|----|------|------|-------|------|--------------|------|-----|-------|------|------------|-----|-----|-----|-----|------------|
| | Val | Ala | Glu | Leu | | Ile | Ser | Pro | Ser | | Asn | Ala | Val | Val | |
| 5 | Thr | Asn | Leu | Leu | Pro 65 | Gly | Thr | Glu | Tyr | Val | Val | Ser | Val | Ser | Ser 75 |
| | Val | Tyr | Glu | Gln | | Glu | Ser | Thr | Pro | Leu 85 | Arg | Gly | Arg | Gln | Lys 90 |
| | Thr | Gly | Leu | Asp | | Pro | Thr | Gly | Ile | Asp 100 | Phe | Ser | Asp | Ile | Thr 105 |
| 10 | Ala | Asn | Ser | Phe | Thr 110 | Val | His | Trp | Ile | Ala 115 | Pro | Arg | Ala | Thr | Ile 120 |
| | Thr | Gly | туr | Arg | | Arg | His | His | Pro | | His | Phe | Ser | Gly | Arg 135 |
| | Pro | Arg | Glu | Asp | | Val | Pro | His | Ser | Arg 145 | Asn | Ser | Ile | Thr | Leu 150 |
| 15 | Thr | Asn | Leu | Thr | | Gly | Thr | Glu | Tyr | | Val | Ser | Ile | Val | |
| | Leu | Asn | Gly | Arg | | Glu | Ser | Pro | Leu | | Ile | Gly | Gln | Gln | |
| | Thr | Val | Ser | Asp | | Pro | Arg | Asp | Leu | | Val | Val | Ala | Ala | |
| 20 | Pro | Thr | Ser | Leu | | Ile | Ser | Trp | Asp | | Pro | Ala | Val | Thr | |
| | Arg | Tyr | Tyr | Arg | | Thr | Tyr | Gly | Glu | | Gly | Gly | Asn | Ser | |
| 25 | Val | Gln | Glu | Phe | | Val | Pro | Gly | Ser | | Ser | Thr | Ala | Thr | |
| | Ser | Gly | Leu | Lys | | Gly | Val | Asp | Tyr | | Ile | Thr | Val | Tyr | |
| | Val | Thr | Gly | Arg | | Asp | Ser | Pro | Ala | | Ser | Lys | Pro | Ile | |
| 30 | Ile | Asn | Tyr | Arg | | Glu | Ile | Asp | Lys | | Ser | Gln | Met | | |
| | | | | | FOR ARAC' | | | NO: 8 | 8: | | | | | | |
| 35 | (A) | LEN | GTH: | 279 | aci | | | - • | | | | | | | |
| | (C) | STR | ANDE | DNES | S: s | ingl | е | | | | | | | | |
| | (ii) |) MO | LECU: | LE T | YPE: | pep | | SEQ : | ID N | 0:8 | : | | | | |
| 40 | , , | | | | | | | | | | | Asp | Thr | Met | Ara |
| | 1 | | | | 5 | | | | | 10 | | | Asn | | 15 |
| | | | | | 20 | | | | | 25 | | | Ala | | 30 |
| 45 | | | | | 35 | | | | | 40 | | | Asn | | 45 |
| | | | | | 50 | _ | | | | 55 | | | | | 60 |
| | | | | | 65 | | | | | 70 | | | Tyr | | 75 |
| 50 | | | | | 80 | | _ | | _ | 85 | | | Gly | | 90 |
| | | | | _ | 95 | | | | | 100 | | | Asn | | 105 |
| | Thr | Val | His | Trp | Ile | Ala | Pro | Arg | Ala | Thr | Ile | Thr | Gly | Tyr | Arg |
| | | | | | | | | | | | | | | | |

| | Ile | Arg | His | His | 110 Pro | Glu | His | Phe | Ser | 115 Gly | Arg | Pro | Arg | Glu | 120 Asp |
|----------------|--|---|---|--|--|--|--|---|---|---|---|---|--|---|---|
| 5 | Arg | Val | Pro | His | 125 Ser | Arg | Asn | Ser | Ile | 130 Thr | Leu | Thr | Asn | Leu | 135 Thr |
| | Pro | Gly | Thr | Glu | | Val | Val | Ser | Ile | | Ala | Leu | Asn | Gly | |
| | Glu | Glu | Ser | Pro | | Leu | Ile | Gly | Gln | | Ser | Thr | Val | Ser | |
| 10 | Val | Pro | Arg | Asp | 170 Leu 185 | Glu | Val | Val | Ala | 175 Ala 190 | Thr | Pro | Thr | Ser | 180 Leu 195 |
| | Leu | Ile | Ser | Trp | | Ala | Pro | Ala | Val | | Val | Arg | Tyr | Tyr | |
| | Ile | Thr | Tyr | Gly | | Thr | Gly | Gly | Asn | | Pro | Val | Gln | Glu | |
| 15 | Thr | Val | Pro | Gly | | Lys | Ser | Thr | Ala | | Ile | Ser | Gly | Leu | |
| | Pro | Gly | Val | Asp | Tyr 245 | Thr | Ile | Thr | Val | Tyr 250 | Ala | Val | Thr | Gly | Arg 255 |
| 20 | Gly | Asp | Ser | Pro | Ala 260 | Ser | Ser | Lys | Pro | Ile 265 | Ser | Ile | Asn | Tyr | Arg 270 |
| | Thr | Glu | Ile | Asp | Lys 275 | Pro | Ser | Gln | Met | | | | | | |
| 25 | (i) (A) (B) (C) | INFO SEQU LENO TYPE STRA | JENCI STH: E: ar ANDEI | E CHA 474 mino ONESS | acio | renīs i ingle | STICS | | 9: | | | | | | |
| | (ii |) MOI | LECUI | | YPE: | pept | | SEO 1 | ED NI | ۵ ، د | • | | | | |
| 30 | (ii (xi |) MOI) SE(| LECUI QUENC | LE TY | PE: ESCR | pept IPTIC | ON: S | | | | | Asn | Ile | Glv | Pro |
| 30 | (ii) (xi) Ala 1 |) MOI) SE(Val | LECUI QUENC Pro | LE TY CE DI Pro | YPE: ESCRI Pro 5 | pept IPTIC Thr | Asp | Leu | Arg | Phe 10 | Thr | | | - | 15 |
| 30 | (ii) (xi) Ala 1 Asp |) MOI) SE(| DECUI QUENC Pro Met | LE TY CE DE Pro Arg | YPE: ESCRI Pro 5 Val 20 | pept IPTIC Thr Thr | ON: S Asp Trp | Leu Ala | Arg Pro | Phe 10 Pro 25 | Thr Pro | Ser | Ile | Asp | 15 Leu 30 |
| | (ii) (xi) Ala 1 Asp |) MOI) SE(Val Thr | Pro Met Phe | EE TY CE DE Pro Arg Leu | Pro 5 Val 20 Val 35 | pept IPTIC Thr Thr Arg | ON: S Asp Trp Tyr | Leu Ala Ser | Arg Pro Pro | Phe 10 Pro 25 Val 40 | Thr Pro Lys | Ser Asn | Ile Glu | Asp Glu | 15 Leu 30 Asp 45 |
| | (ii) (xi) Ala 1 Asp Thr | MOI SE(Val Thr Asn | DECUI QUENC Pro Met Phe Glu | CE TY Pro Arg Leu Leu | PPE: ESCRI Pro 5 Val 20 Val 35 Ser 50 Pro | pept IPTIC Thr Arg Ile | Asp Trp Tyr Ser | Leu Ala Ser Pro | Arg Pro Pro Ser | Phe 10 Pro 25 Val 40 Asp 55 Val | Thr Pro Lys Asn | Ser Asn Ala | Ile Glu Val | Asp Glu Val | 15 Leu 30 Asp 45 Leu 60 Ser |
| | (ii) (xi) Ala 1 Asp Thr Val | MOI SEQ Val Thr Asn | Pro Met Phe Glu Leu | Pro Arg Leu Leu Leu | PPE: ESCRI Pro 5 Val 20 Val 35 Ser 50 Pro 65 His | pept IPTIC Thr Thr Arg Ile Gly | Asp Trp Tyr Ser | Leu Ala Ser Pro Glu | Arg Pro Pro Ser Tyr | Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu | Thr Pro Lys Asn Val | Ser Asn Ala Ser | Ile Glu Val Val | Asp Glu Val Ser | 15 Leu 30 Asp 45 Leu 60 Ser 75 Lys |
| 35 | (ii) (xi) Ala 1 Asp Thr Val Thr | MOI SE(Val Thr Asn Ala Asn | Pro Met Phe Glu Leu Glu | Pro Arg Leu Leu Gln | PPE: Pro 5 Val 20 Val 35 Ser 50 Pro 65 His 80 Ser | pept IPTIC Thr Thr Arg Ile Gly | Asp Trp Tyr Ser Thr | Leu Ala Ser Pro Glu Thr | Arg Pro Pro Ser Tyr Pro | Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp | Thr Pro Lys Asn Val | Ser Asn Ala Ser Gly | Ile Glu Val Val Arg | Asp Glu Val Ser | 15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr |
| 35 | (ii) (xi) Ala 1 Asp Thr Val Thr | MOI) SEG Val Thr Asn Ala Asn Tyr | Pro Met Phe Glu Leu Glu Leu | Pro Arg Leu Leu Leu Gln Asp | PE: Pro 5 Val 20 Val 35 Ser 50 Pro 65 His 80 Ser 95 Thr | Thr Thr Arg Ile Gly Glu Pro | Asp Trp Tyr Ser Thr Ser | Leu Ala Ser Pro Glu Thr Gly | Arg Pro Pro Ser Tyr Pro Ile | Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp 100 Ala | Thr Pro Lys Asn Val Arg | Ser Asn Ala Ser Gly Ser | Ile Glu Val Val Arg Asp | Asp Glu Val Ser Gln Ile | 15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 Ile |
| 35 | (ii) (xi) Ala 1 Asp Thr Val Thr Val Thr | MOI SEC Val Thr Asn Ala Asn Tyr | Pro Met Phe Glu Leu Glu Leu Ser | Pro Arg Leu Leu Leu Gln Asp | PE: ESCRI Pro 5 Val 20 Val 35 Ser 50 Pro 65 His 80 Ser 95 Thr 110 | Thr Thr Arg Ile Gly Glu Pro Val | Asp Trp Tyr Ser Thr Ser Thr | Leu Ala Ser Pro Glu Thr Gly Trp | Arg Pro Pro Ser Tyr Pro Ile Ile | Phe 10 Pro 25 Val 40 Asp 55 Val R5 Asp 100 Ala 115 Glu | Thr Pro Lys Asn Val Arg Phe | Ser Asn Ala Ser Gly Ser Arg | Ile Glu Val Val Arg Asp | Asp Glu Val Ser Gln Ile | 15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 Ile 120 Arg |
| <i>35</i> | (ii) (xi) Ala 1 Asp Thr Val Thr Ala Thr | MOI) SEG Val Thr Asn Ala Asn Tyr Gly Asn | Pro Met Phe Glu Leu Glu Leu Tyr | Pro Arg Leu Leu Gln Asp Phe Arg | PE: ESCRI Pro 5 Val 20 Val 35 Ser 50 Pro 65 His ser 95 Thr 110 Ile 125 | Thr Thr Arg Ile Gly Glu Pro Val Arg | Asp Trp Tyr Ser Thr Ser Thr His | Leu Ala Ser Pro Glu Thr Gly Trp His | Arg Pro Pro Ser Tyr Pro Ile Ile Pro | Phe 10 Pro 25 Val 40 Asp 55 Val 100 Ala 115 Glu 130 Arg | Thr Pro Lys Asn Val Arg Phe Pro His | Ser Asn Ala Ser Gly Ser Arg Phe | Ile Glu Val Val Arg Asp Ala Ser | Asp Glu Val Ser Gln Ile Thr | 15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 Ile 120 Arg 135 Leu |
| <i>35</i> | (ii) (xi) Ala 1 Asp Thr Val Thr Ala Thr Pro | MOI SEG Val Thr Asn Ala Asn Tyr Gly Asn | Pro Met Phe Glu Leu Glu Leu Tyr Glu | Pro Arg Leu Leu Gln Asp Phe Arg | PE: ESCRIPTO Val 20 Val 35 Ser 50 Pro 65 80 Pro 110 11e 125 Arg | Thr Thr Arg Ile Gly Glu Pro Val Arg Val | Asp Trp Tyr Ser Thr Ser Thr His | Leu Ala ser Pro Glu Thr Gly Trp His | Arg Pro Pro Ser Tyr Pro Ile Ile Pro Ser | Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp 100 Ala 115 Glu 130 Arg | Thr Pro Lys Asn Val Arg Phe Pro His | Ser Asn Ala Ser Gly Ser Arg Phe | Ile Glu Val Val Arg Asp Ala Ser | Asp Glu Val Ser Gln Ile Thr Gly | 15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 Ile 120 Arg 135 Leu 150 |
| 35 40 45 | (ii) (xi) Ala 1 Asp Thr Val Thr Ala Thr Ala Thr | MOI SEG Val Thr Asn Ala Asn Tyr Gly Asn Gly | Pro Met Phe Glu Leu Glu Leu Ser Tyr Glu Leu | LE TYCE DE Pro Arg Leu Leu Gln Asp Phe Arg Asp Thr | PE: ESCRIPTO TO SERVICE STATE OF THE SERVICE STATE | Thr Thr Arg Ile Gly Pro Val Arg Val Gly | Asp Trp Tyr Ser Thr Ser His His Pro | Leu Ala Ser Pro Glu Thr Gly Trp His His | Arg Pro Pro Ser Tyr Pro Ile Ile Pro Ser Tyr | Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp 115 Glu 130 Arg 145 Val 160 | Thr Pro Lys Asn Val Arg Phe Pro His Asn | Ser Asn Ala Ser Gly Ser Arg Phe Ser Ser | Ile Glu Val Val Arg Asp Ala Ser Ile | Asp Glu Val Ser Gln Ile Thr Gly Thr | 15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 11e 120 Arg 135 Leu 150 Ala 165 |

| | | | | | 185 | | | | | 190 | | | | | 195 |
|----|-------|-------------|------------|------------|------------|-----------|------------|--------------|-----------|------------|-----------|------------|------------|-------------|------------|
| | Pro | Thr | Ser | Leu | | Ile | Ser | Trp | Asp | | Pro | Ala | Val | Thr | |
| | Ara | Tyr | Tvr | Ara | 200 Tle | Thr | Tvr | Glv | Glu | 205 Thr | Glv | Glv | Asn | Ser | 210 Pro |
| 5 | 9 | | -1- | 1119 | 215 | | - 3 - | -1 | 014 | 220 | <u>-1</u> | OL, | | DOL | 225 |
| | Val | Gln | Glu | Phe | | Val | Pro | Gly | Ser | | Ser | Thr | Ala | Thr | |
| | 502 | C1., | Lou | Tva | 230 | Gly | 77-1 | 7 50 | Tur | 235 | Tla | Thr | 77-1 | Пол | 240 |
| | SeT | GIY | Leu | пуъ | 245 | Giy | vaı | wsb | тут | 250 | 116 | 1111 | Val | TÄT | 255 |
| 10 | Val | Thr | Gly | Arg | _ | Asp | Ser | Pro | Ala | | Ser | Lys | Pro | Ile | |
| | T10 | n an | m | 7) 20 00 | 260 | ~1., | T10 | 7 an | Tuc | 265 Pro | Cor | Cln | 7 an | C1., | 270 |
| | TTE | ASII | тут | Arg | 275 | Glu | 116 | Asp | пуъ | 280 | er. | GIII | ASII | Giu | 285 |
| | Leu | Asn | Gln | Pro | Thr | Asp | Asp | Ser | Cys | Phe | Asp | Pro | Tyr | Thr | Val |
| 15 | G | T7.2 - | m | 7.7. | 290 | ~1 | 70 | C1 | TT | 295 | 7 | Mat | C | C1 | 300 |
| - | ser | птъ | туг | AIa | 305 | Gly | ASP | Giu | .rrb | 310 | ALG | Met | per | GIU | 315 |
| | Gly | Phe | Lys | Leu | Leu | Cys | Gln | Cys | Leu | Gly | Phe | Gly | Ser | Gly | His |
| | _, | | - | _ | 320 | | | | ~ | 325 | | | ~ 1 | **- 3 | 330 |
| •• | Phe | Arg | Cys | Asp | 335 | Ser | Arg | Trp | Cys | H15 | Asp | Asn | GIA | vaı | Asn 345 |
| 20 | Tyr | Lys | Ile | Gly | | Lys | Trp | Asp | Arg | | Gly | Glu | Asn | Gly | |
| | 16-1- | > | ~ | ~ | 350 | ~ | . | | 7 | 355 | ~ | ~1 | ~1 | D). | 360 |
| | мет | мет | ser | Cys | 365 | Cys | ьeu | СТА | Asn | 370 | туѕ | GTÀ | GIU | Pne | шуs 375 |
| | Cys | Asp | Pro | His | | Ala | Thr | Cys | Tyr | | Asp | Gly | Lys | Thr | |
| 25 | | 1 | ~ 1 | ~ 1 | 380 | | a 1 | ~ | 01 | 385 | . | ~ 1 | 77- | - 1- | 390 |
| | Hls | val | GTA | GIU | GIN 395 | Trp | GIN | гла | GLU | 1yr 400 | ьeu | СТА | Ата | TTE | 405 |
| | Ser | Cys | Thr | Cys | | Gly | Gly | Gln | Arg | | Trp | Arg | Cys | Asp | |
| | _ | | _ | _ | 410 | | | _ | _ | 415 | ~ 3 | ~- | | | 420 |
| 30 | Cys | Arg | Arg | Pro | 425 | Gly | GIU | Pro | ser | 430 | GIU | GTA | Thr | Thr | 435 |
| | Gln | Ser | Tyr | Asn | | Tyr | Ser | Gln | Arg | | His | Gln | Arg | Thr | - |
| | m1 | | ** 1 | | 440 | _ | | ~ 3 . | | 445 | | _ | _ | _ | 450 |
| | Thr | Asn | vaı | Asn | 455 | Pro | тте | GIU | Cys | 460 | Met | Pro | ьeu | Asp | Val 465 |
| 35 | Gln | Ala | Asp | Arg | | Asp | Ser | Arg | Glu | | | | | | |
| | | | | | 470 | | | | | | | | | | |
| | (2) | TNF | יבשמר | PTON. | FOR | SEO | א חד | ٠ | 10- | | | | | | |
| | | | | | | reri: | | | | | | | | | |
| | (A) | | STH: | | | | | | | | | | | | |
| 40 | (B) | | | nino | | | | | | | | | | | |
| | (C) | | | | | ingle | € | | | | | | | | |
| | (D) | TOP | JLOG: | Y: 1: | ınear | r | | | | | | | | | |

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

55

45

| | | | | | 65 | | | | | 70 | | | | | 75 |
|----|-----------|-------|--------|------------|------------|------|------|---------------|-------|--------------------|-----|-----|-----|-----|------------|
| | Pro | Gly | Leu | Glu | Tyr 80 | Asn | Val | Ser | Val | Tyr 85 | Thr | Val | Lys | Asp | Asp 90 |
| 5 | Lys | Glu | Ser | Val | Pro 95 | Ile | Ser | Asp | Thr | Ile 100 | Ile | Pro | Ala | Val | Pro 105 |
| | Pro | Pro | Thr | Asp | Leu 110 | Arg | Phe | Thr | Asn | Ile 115 | Gly | Pro | Asp | Thr | Met 120 |
| | Arg | Val | Thr | Trp | Ala 125 | Pro | Pro | Pro | Ser | Ile 130 | Asp | Leu | Thr | Asn | Phe 135 |
| 10 | Leu | Val | Arg | Tyr | Ser 140 | Pro | Val | Lys | Asn | Glu 145 | Glu | Asp | Val | Ala | Glu 150 |
| | Leu | Ser | Ile | Ser | Pro 155 | Ser | Asp | Asn | Ala | Val 160 | Val | Leu | Thr | Asn | Leu 165 |
| | Leu | Pro | Gly | Thr | Glu 170 | Tyr | Val | Val | Ser | Val 175 | Ser | Ser | Val | Tyr | Glu 180 |
| 15 | Gln | His | Glu | Ser | Thr 185 | Pro | Leu | Arg | Gly | Arg 190 | Gln | Lys | Thr | Gly | |
| | Asp | Ser | Pro | Thr | | Ile | Asp | Phe | Ser | | Ile | Thr | Ala | Asn | |
| 00 | Phe | Thr | Val | His | | Ile | Ala | Pro | Arg | | Thr | Ile | Thr | Gly | |
| 20 | Arg | Ile | Arg | His | His 230 | Pro | Glu | His | Phe | Ser 235 | Gly | Arg | Pro | Arg | Glu 240 |
| | Asp | Arg | Val | Pro | His 245 | Ser | Arg | Asn | Ser | Ile 250 | Thr | Leu | Thr | Asn | Leu 255 |
| 25 | Thr | Pro | Gly | Thr | Glu 260 | Tyr | Val | Val | Ser | Ile 265 | Val | Ala | Leu | Asn | Gly 270 |
| | Arg | Glu | Glu | Ser | Pro 275 | Leu | Leu | Ile | Gly | Gln 280 | Gln | Ser | Thr | Val | Ser 285 |
| | Asp | Val | Pro | Arg | Asp 290 | Leu | Glu | Val | Val | Ala 295 | Ala | Thr | Pro | Thr | Ser 300 |
| 30 | Leu | Leu | Ile | Ser | Trp 305 | Asp | Ala | Pro | Ala | Val 310 | Thr | Val | Arg | Tyr | Tyr 315 |
| | Arg | Ile | Thr | Tyr | Gly 320 | Glu | Thr | Gly | Gly | Asn 325 | Ser | Pro | Val | Gln | Glu 330 |
| | Phe | Thr | Val | Pro | Gly 335 | Ser | Lys | Ser | Thr | Ala 340 | Thr | Ile | Ser | Gly | Leu 345 |
| 35 | Lys | Pro | Gly | Val | Asp 350 | Tyr | Thr | Ile | Thr | Val 355 | Tyr | Ala | Val | Thr | Gly 360 |
| | Arg | Gly | Asp | Ser | Pro 365 | Ala | Ser | Ser | Lys | Pro 370 | Ile | Ser | Ile | Asn | Tyr 375 |
| | Arg | Thr | Glu | Ile | Asp 380 | Lys | Pro | Ser | Gln | Met 385 | | | | | |
| 40 | (2) | T3757 | 201476 | T.C.1 | HOD | 0.00 | TD 1 | 70 - 7 | 11. | | | | | | |
| | | INF | | | | | | | 11: | | | | | | |
| | (A) | LEN | GTH: | 549 | | | | | | | | | | | |
| | | TYPI | | | | | _ | | | | | | | | |
| 45 | | TOP | | | | _ | = | | | | | | | | |
| | |) MOI | | | | | | SEO T | או חד |) - 1 ⁻ | 1 • | | | | |
| | \ | , 22 | ~ | <i>-</i> 1 | | (| -11. | - <u></u> 2 - | | -• ±. | - • | | ~ | | |
| 50 | 1 | Thr | _ | | 5 | | | | | 10 | | _ | | | 15 |
| | Val | Thr | Trp | Ala | Pro 20 | Pro | Pro | Ser | Ile | Asp 25 | Leu | Thr | Asn | Phe | Leu 30 |
| | Val | Arg | Tyr | Ser | Pro | Val | Lys | Asn | Glu | Glu | Asp | Val | Ala | Glu | Leu |

| | | | | _ | 35 | _ | _ | | | 40 | _ | | _ | _ | 45 |
|----|-----|-----|-----|-----|------------|-----|-----|-----|------|------------|-----|-----|-----|-----|------------|
| | Ser | He | Ser | Pro | Ser 50 | Asp | Asn | Ala | Va⊥ | 55 | Leu | Thr | Asn | Leu | Leu 60 |
| 5 | Pro | Gly | Thr | Glu | Tyr 65 | Val | Val | Ser | Val | Ser 70 | Ser | Val | Tyr | Glu | Gln 75 |
| | His | Glu | Ser | Thr | Pro 80 | Leu | Arg | Gly | Arg | Gln 85 | Lys | Thr | Gly | Leu | Asp 90 |
| | Ser | Pro | Thr | Gly | | Asp | Phe | Ser | Asp | | Thr | Ala | Asn | Ser | |
| 10 | Thr | Val | His | Trp | Ile | Ala | Pro | Arg | Ala | Thr | Ile | Thr | Gly | Tyr | |
| | Ile | Arg | His | His | | Glu | His | Phe | Ser | | Arg | Pro | Arg | Glu | Asp |
| | Arg | Val | Pro | His | | Arg | Asn | Ser | Ile | | Leu | Thr | Asn | Leu | |
| 15 | Pro | Gly | Thr | Glu | 140 Tyr | Val | Val | Ser | ·Ile | 145 Val | Ala | Leu | Asn | Gly | 150 Arg |
| | Glu | Glu | Ser | Pro | 155 Leu | Leu | Ile | Gly | Gln | 160 Gln | Ser | Thr | Val | Ser | 165 Asp |
| | Val | Pro | Arg | Asp | 170 Leu | Glu | Val | Val | Ala | 175 Ala | Thr | Pro | Thr | Ser | 180 Leu |
| 20 | Leu | Ile | Ser | Trp | 185 Asp | Ala | Pro | Ala | Val | 190 Thr | Val | Ara | Tvr | Tyr | 195 Ara |
| | | | | | 200 | | | | | 205 | | _ | _ | Glu | 210 |
| | | | Ī | | 215 | | _ | - | | 220 | | | | | 225 |
| 25 | | | | | 230 | | | | | 235 | | | | Leu | 240 |
| | Pro | Gly | Val | Asp | Tyr 245 | Thr | Ile | Thr | Val | Tyr 250 | Ala | Val | Thr | Gly | Arg 255 |
| | Gly | Asp | Ser | Pro | Ala 260 | Ser | Ser | Lys | Pro | Ile 265 | Ser | Ile | Asn | Tyr | Arg 270 |
| 30 | Thr | Glu | Ile | Asp | Lys 275 | Pro | Ser | Met | Ala | Ile 280 | Pro | Ala | Pro | Thr | Asp 285 |
| | Leu | Lys | Phe | Thr | Gln 290 | Val | Thr | Pro | Thr | Ser 295 | Leu | Ser | Ala | Gln | Trp 300 |
| | Thr | Pro | Pro | Asn | | Gln | Leu | Thr | Gly | | Arg | Val | Arg | Val | |
| 35 | Pro | Lys | Glu | Lys | | Gly | Pro | Met | Lys | | Ile | Asn | Leu | Ala | Pro |
| | Asp | Ser | Ser | Ser | Val | Val | Val | Ser | Gly | Leu | Met | Val | Ala | Thr | |
| | Tyr | Glu | Val | Ser | | Tyr | Ala | Leu | Lys | | Thr | Leu | Thr | Ser | |
| 40 | Pro | Ala | Gln | Gly | | Val | Thr | Thr | Leu | | Asn | Val | Ser | Pro | |
| | Arg | Arg | Ala | Arg | 365 Val | Thr | Asp | Ala | Thr | 370 Glu | Thr | Thr | Ile | Thr | 375 Ile |
| | Ser | Trp | Arg | Thr | 380 Lys | Thr | Glu | Thr | Ile | 385 Thr | Gly | Phe | Gln | Val | 390 Asp |
| 45 | | | | | 395 | | | | | 400 | | | | Ile | 405 |
| | | | | | 410 | | | | | 415 | | | | Gly | 420 |
| | | | | | 425 | | | | | 430 | | | - | _ | 435 |
| 50 | | | | | 440 | | | | | 445 | _ | | | Arg | 450 |
| | | | | | 455 | _ | | | | 460 | | _ | | Pro | 465 |
| | Asn | Leu | Arg | Phe | Leu | Ala | Thr | Thr | Pro | Asn | Ser | Leu | Leu | Val | Ser |

| | | 01 | D | D | 470 | חות | 7~~ | Tlo | mh w | 475 | Птт | Tlo | Tle | Luc | 480 |
|----|----------------------------------|--|---|--|---------------------------------------|------------------------|-------|-----|------|------------|-----|-----|-----|-----|------------|
| | | | | | 485 | | | | | 490 | | Ile | | | 495 |
| 5 | Glu | Lys | Pro | Gly | Ser 500 | Pro | Pro | Arg | Glu | Val 505 | Val | Pro | Arg | Pro | Arg 510 |
| | Pro | Gly | Val | Thr | Glu 515 | Ala | Thr | Ile | Thr | Gly 520 | Leu | Glu | Pro | Gly | Thr 525 |
| | Glu | Tyr | Thr | Ile | | Val | Ile | Ala | Leu | | Asn | Asn | Gln | Lys | |
| 10 | Glu | Pro | Leu | Ile | Gly 545 | Arg | Lys | Lys | Thr | | | | | | |
| 15 | (A) (B) (C) (D) (ii) | INFO SEQU LENO TYPH STRA TOPO MOI) SEQ | JENCH STH: E: an ANDEI DLOGY LECUI | E CHA 422 nino ONESS (: 1: | acio acio s: si inea (PE: | ingle ingle pept | erics | S: | | D: 12 | 2: | | | | |
| 20 | | | | | | | | | | | | _ | | | _ |
| | Pro | Thr | Asp | Leu | Arg 5 | Phe | Thr | Asn | Ile | Gly 10 | Pro | Asp | Thr | Met | Arg 15 |
| | Val | Thr | Trp | Ala | Pro 20 | Pro | Pro | Ser | Ile | Asp 25 | Leu | Thr | Asn | Phe | Leu 30 |
| 25 | Val | Arg | Tyr | Ser | Pro 35 | Val | Lys | Asn | Glu | Glu 40 | Asp | Val | Ala | Glu | Leu 45 |
| | Ser | Ile | Ser | Pro | | Asp | Asn | Ala | Val | | Leu | Thr | Asn | Leu | |
| | Pro | Gly | Thr | Glu | Tyr 65 | Val | Val | Ser | Val | Ser 70 | Ser | Val | Tyr | Glu | Gln 75 |
| 30 | His | Glu | Ser | Thr | Pro 80 | Leu | Arg | Gly | Arg | Gln 85 | Lys | Thr | Gly | Leu | Asp 90 |
| | Ser | Pro | Thr | Gly | Ile 95 | Asp | Phe | Ser | Asp | Ile 100 | Thr | Ala | Asn | Ser | Phe 105 |
| | Thr | Val | His | Trp | Ile 110 | Ala | Pro | Arg | Ala | Thr 115 | Ile | Thr | Gly | Tyr | Arg 120 |
| 35 | Ile | Arg | His | His | Pro 125 | Glu | His | Phe | Ser | Gly 130 | Arg | Pro | Arg | Glu | Asp 135 |
| | Arg | Val | Pro | His | Ser 140 | Arg | Asn | Ser | Ile | Thr 145 | Leu | Thr | Asn | Leu | Thr 150 |
| | Pro | Gly | Thr | Glu | Tyr 155 | Val | Val | Ser | Ile | Val 160 | Ala | Leu | Asn | Gly | Arg 165 |
| 40 | Glu | Glu | Ser | Pro | Leu 170 | Leu | Ile | Gly | Gln | Gln 175 | Ser | Thr | Val | Ser | Asp 180 |
| | Val | Pro | Arg | Asp | Leu 185 | Glu | ۷al | Val | Ala | Ala 190 | Thr | Pro | Thr | Ser | Leu 195 |
| 45 | Leu | Ile | Ser | Trp | Asp 200 | Ala | Pro | Ala | Val | Thr 205 | Val | Arg | Tyr | Tyr | Arg 210 |
| 45 | Ile | Thr | Tyr | Gly | Glu 215 | Thr | Gly | Gly | Asn | Ser 220 | Pro | Val | Gln | Glu | Phe 225 |
| | Thr | Val | Pro | Gly | Ser 230 | Lys | Ser | Thr | Ala | Thr 235 | Ile | Ser | Gly | Leu | Lys 240 |
| 50 | Pro | Gly | Val | Asp | Tyr 245 | Thr | Ile | Thr | Val | Tyr 250 | Ala | Val | Thr | Gly | Arg 255 |
| | Gly | Asp | Ser | Pro | Ala 260 | Ser | Ser | Lys | Pro | Ile 265 | Ser | Ile | Asn | Tyr | Arg 270 |
| | Thr | Glu | Ile | Asp | | Pro | Ser | Met | Ala | | Glu | Gly | Leu | Asn | |

| | Pro | Thr | Asp | Asp | 275 Ser 290 | Cys | Phe | Asp | Pro | 280 Tyr 295 | Thr | Val | Ser | His | 285 Tyr 300 |
|----------------|--|---|---|---|---|---|---|---|--|---|---|---|--|---|---|
| 5 | Ala | Val | Gly | Asp | | Trp | Glu | Arg | Met | | Glu | Ser | Gly | Phe | |
| | Leu | Leu | Cys | Gln | | Leu | Gly | Phe | Gly | . — . | Gly | His | Phe | Arg | |
| | Asp | Ser | Ser | Arg | | Cys | His | Asp | Asn | | Val | Asn | Tyr | Lys | |
| 10 | Gly | Glu | Lys | Trp | | Arg | Gln | Gly | Glu | | Gly | Gln | Met | Met | |
| | Cys | Thr | Cys | Leu | | Asn | Gly | Lys | Gly | | Phe | Lys | Cys | Asp | |
| | His | Glu | Ala | Thr | | Tyr | Asp | Asp | Gly | | Thr | Tyr | His | Val | |
| 15 | Glu | Gln | Trp | Gln | | Glu | Tyr | Leu | Gly | | Ile | Cys | Ser | Cys | |
| | Cys | Phe | Gly | Gly | | Arg | Gly | Trp | Arg | | Asp | Asn | Cys | Arg | |
| 20 | Pro | Gly | | | | | | | | | | | | | |
| 20 | (i) (A) | SEQU LENC TYPE | JENCI STH: E: ar | rion E CHA 332 mino ONES | ARAC: | reri: | STICS | | 13: | | | | | | |
| 25 | (ii) | MOI | LECUI | Y: l: LE T: CE DI | PE: | pept | | SEQ I | ID NO |): 1: | 3: | | | | |
| | _ | | | | | | | | | | | | | | |
| 20 | 1 | | _ | | 5 | | | | | 10 | | Asp | | | 15 |
| 30 | 1 | | _ | | 5 | | | | | 10 | | Asp Thr | | | 15 |
| 30 | 1 Val Val | Thr Arg | Trp Tyr | Ala Ser | 5 Pro 20 Pro 35 | Pro Val | Pro Lys | Ser Asn | Ile Glu | 10 Asp 25 Glu 40 | Leu Asp | Thr Val | Asn Ala | Phe Glu | 15 Leu 30 Leu 45 |
| <i>30</i> | 1 Val Val Ser | Thr Arg Ile | Trp Tyr Ser | Ala Ser Pro | 5 Pro 20 Pro 35 Ser 50 | Pro Val Asp | Pro Lys Asn | Ser Asn Ala | Ile Glu Val | 10 Asp 25 Glu 40 Val 55 | Leu Asp Leu | Thr Val Thr | Asn Ala Asn | Phe Glu Leu | 15 Leu 30 Leu 45 Leu 60 |
| | 1 Val Val Ser | Thr Arg Ile Gly | Trp Tyr Ser Thr | Ala Ser Pro Glu | 5 Pro 20 Pro 35 Ser 50 Tyr 65 | Pro Val Asp Val | Pro Lys Asn Val | Ser Asn Ala Ser | Ile Glu Val Val | 10 Asp 25 Glu 40 Val 55 Ser 70 | Leu Asp Leu Ser | Thr Val Thr Val | Asn Ala Asn Tyr | Phe Glu Leu Glu | 15 Leu 30 Leu 45 Leu 60 Gln 75 |
| | 1 Val Val Ser | Thr Arg Ile Gly | Trp Tyr Ser Thr | Ala Ser Pro Glu | 5 Pro 20 Pro 35 Ser 50 Tyr 65 | Pro Val Asp Val | Pro Lys Asn Val | Ser Asn Ala Ser | Ile Glu Val Val | 10 Asp 25 Glu 40 Val 55 Ser 70 | Leu Asp Leu Ser | Thr Val Thr | Asn Ala Asn Tyr | Phe Glu Leu Glu | 15 Leu 30 Leu 45 Leu 60 Gln 75 |
| | 1 Val Val Ser Pro | Thr Arg Ile Gly Glu | Trp Tyr Ser Thr | Ala Ser Pro Glu Thr | 5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 | Pro Val Asp Val Leu | Pro Lys Asn Val Arg | Ser Asn Ala Ser Gly | Ile Glu Val Val Arg | 10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 | Leu Asp Leu Ser Lys | Thr Val Thr Val | Asn Ala Asn Tyr Gly | Phe Glu Leu Glu Leu | 15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 |
| 35 | 1 Val Val Ser Pro His Ser Thr | Thr Arg Ile Gly Glu Pro Val | Trp Tyr Ser Thr Ser Thr | Ala Ser Pro Glu Thr Gly Trp | 5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 95 Ile 110 | Pro Val Asp Val Leu Asp | Pro Lys Asn Val Arg Phe | Ser Asn Ala Ser Gly Ser Arg | Ile Glu Val Val Arg Asp | 10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr | Leu Asp Leu Ser Lys Thr | Thr Val Thr Val Thr Ala | Asn Ala Asn Tyr Gly Asn Gly | Phe Glu Leu Glu Leu Ser Tyr | 15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105 Arg 120 |
| 35 | Val Val Ser Pro His Ser Thr | Thr Arg Ile Gly Glu Pro Val Arg | Trp Tyr Ser Thr Ser Thr His | Ala Ser Pro Glu Thr Gly Trp | 5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 95 Ile 110 Pro 125 | Pro Val Asp Val Leu Asp Ala Glu | Pro Lys Asn Val Arg Phe Pro | Ser Asn Ala Ser Gly Ser Arg | Ile Glu Val Val Arg Asp Ala Ser | 10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130 | Leu Asp Leu Ser Lys Thr Ile Arg | Thr Val Thr Val Thr Ala Thr | Asn Ala Asn Tyr Gly Asn Gly Arg | Phe Glu Leu Glu Leu Ser Tyr | 15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105 Arg 120 Asp 135 |
| 35 | Val Val Ser Pro His Ser Thr Ile | Thr Arg Ile Gly Glu Pro Val Arg | Trp Tyr Ser Thr Ser Thr His | Ala Ser Pro Glu Thr Gly Trp His | 5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 110 Pro 125 Ser 140 | Pro Val Asp Val Leu Asp Ala Glu Arg | Pro Lys Asn Val Arg Phe Pro His Asn | Ser Asn Ala Ser Gly Ser Arg Phe | Ile Glu Val Val Arg Asp Ala Ser | 10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130 Thr 145 | Leu Asp Leu Ser Lys Thr Ile Arg | Thr Val Thr Val Thr Ala Thr Pro | Asn Ala Asn Tyr Gly Asn Gly Arg | Phe Glu Leu Glu Leu Ser Tyr Glu Leu | 15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105 Arg 120 Asp 135 Thr 150 |
| <i>35</i> | Val Val Ser Pro His Ser Thr Ile | Thr Arg Ile Gly Glu Pro Val Arg | Trp Tyr Ser Thr Ser Thr His | Ala Ser Pro Glu Thr Gly Trp His | 5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 110 Pro 125 Ser 140 | Pro Val Asp Val Leu Asp Ala Glu Arg | Pro Lys Asn Val Arg Phe Pro His | Ser Asn Ala Ser Gly Ser Arg Phe | Ile Glu Val Val Arg Asp Ala Ser | 10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130 Thr 145 | Leu Asp Leu Ser Lys Thr Ile Arg | Thr Val Thr Val Thr Ala Thr | Asn Ala Asn Tyr Gly Asn Gly Arg | Phe Glu Leu Leu Ser Tyr Glu Leu Gly | 15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105 Arg 120 Asp 135 Thr 150 |
| <i>35</i> | Val Val Ser Pro His Ser Thr Ile Arg Pro | Thr Arg Ile Gly Glu Pro Val Arg Val Gly Glu | Trp Tyr Ser Thr Ser Thr His His Pro Thr | Ala Ser Pro Glu Thr Gly Trp His Glu Pro | 5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 110 Pro 125 Ser 140 Tyr 155 Leu 170 | Pro Val Asp Val Leu Asp Ala Glu Arg Val Leu | Pro Lys Asn Val Arg Phe Pro His Asn Val | Ser Asn Ala Ser Gly Ser Arg Phe Ser Ser Gly | Ile Glu Val Val Arg Asp Ala Ser Ile Ile Gln | 10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130 Thr 145 Val 160 Gln 175 | Leu Asp Leu Ser Lys Thr Ile Arg Leu Ala Ser | Thr Val Thr Val Thr Ala Thr Pro Thr Leu | Asn Ala Asn Tyr Gly Asn Gly Arg Arg Asn Val | Phe Glu Leu Glu Leu Ser Tyr Glu Leu Gly Ser | 15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105 Arg 120 Arg 135 Thr 150 Arg 180 |
| <i>35</i> | Val Val Ser Pro His Ser Thr Ile Arg Pro Glu Val | Thr Arg Ile Gly Glu Pro Val Arg Val Gly Glu Pro | Trp Tyr Ser Thr Ser Thr His His Pro Thr Ser | Ala Ser Pro Glu Thr Gly Trp His Glu Pro | 5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 95 Ile 110 Pro 125 Ser 140 Tyr 155 Leu 170 Leu 185 | Pro Val Asp Val Leu Asp Ala Glu Arg Val Leu Glu | Pro Lys Asn Val Arg Phe Pro His Asn Val Ile | Ser Asn Ala Ser Gly Ser Arg Phe Ser Ser Gly Val | Ile Glu Val Val Arg Asp Ala Ser Ile Ile Gln Ala | 10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130 Thr 145 Val 160 Gln 175 Ala 190 | Leu Asp Leu Ser Lys Thr Ile Arg Leu Ala Ser Thr | Thr Val Thr Val Thr Ala Thr Pro Thr Leu Thr | Asn Ala Asn Tyr Gly Asn Gly Arg Arg Asn Val | Phe Glu Leu Ser Tyr Glu Leu Gly Ser | 15 Leu 30 Leu 45 60 Gln 75 Asp 90 Phe 105 Arg 120 Asp 150 Arg 150 Arg 150 Asp 150 Arg 150 A A A A 150 A A A 150 A A A A A 150 A A 150 A A A A A A A A A A A A A A A A A A A |
| 35 40 45 | Val Val Ser Pro His Ser Thr Ile Arg Pro Glu Val | Thr Arg Ile Gly Glu Pro Val Arg Val Gly Glu Pro | Trp Tyr Ser Thr Ser Thr His His Pro Thr Ser | Ala Ser Pro Glu Thr Gly Trp His Glu Pro | 5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 95 Ile 110 Pro 125 Ser 140 Tyr 155 Leu 170 Leu 185 | Pro Val Asp Val Leu Asp Ala Glu Arg Val Leu Glu | Pro Lys Asn Val Arg Phe Pro His Asn Val Ile | Ser Asn Ala Ser Gly Ser Arg Phe Ser Ser Gly Val | Ile Glu Val Val Arg Asp Ala Ser Ile Ile Gln Ala | 10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130 Thr 145 Val 160 Gln 175 Ala 190 | Leu Asp Leu Ser Lys Thr Ile Arg Leu Ala Ser Thr | Thr Val Thr Val Thr Ala Thr Pro Thr Leu | Asn Ala Asn Tyr Gly Asn Gly Arg Arg Asn Val | Phe Glu Leu Ser Tyr Glu Leu Gly Ser | 15 Leu 30 Leu 45 60 Gln 75 Asp 90 Phe 105 Arg 120 Asp 150 Arg 150 Arg 150 Asp 150 Arg 150 A A A A 150 A A A 150 A A A A A 150 A A 150 A A A A A A A A A A A A A A A A A A A |

| | Ile | Thr | Tyr | Gly | Glu 215 | Thr | Gly | Gly | Asn | Ser 220 | Pro | Val | Gln | Glu | Phe 225 |
|----|------------|-------|---------------|-------|------------|--------------|-------|-------|-------|------------|-----|-----|-----|-----|------------|
| - | Thr | Val | Pro | Gly | Ser 230 | Lys | Ser | Thr | Ala | Thr 235 | Ile | Ser | Gly | Leu | Lys 240 |
| 5 | Pro | Gly | Val | Asp | Tyr 245 | Thr | Ile | Thr | Val | Tyr 250 | Ala | Val | Thr | Gly | Arg 255 |
| | Gly | Asp | Ser | Pro | Ala 260 | Ser | Ser | Lys | Pro | Ile 265 | Ser | Ile | Asn | Tyr | Arg 270 |
| 10 | Thr | Glu | Ile | Asp | Lys 275 | Pro | Ser | Met | Ala | Asn 280 | Ser | Asp | Ser | Glu | Cys 285 |
| | Pro | Leu | Ser | His | Asp 290 | Gly | Tyr | Cys | Leu | His 295 | Asp | Gly | Val | Cys | Met 300 |
| | Tyr | Ile | Glu | Ala | Leu 305 | Asp | Lys | Tyr | Ala | Cys 310 | Asn | Cys | Val | Val | Gly 315 |
| 15 | Tyr | Ile | Gly | Glu | Arg 320 | Суѕ | Gln | Tyr | Arg | Asp 325 | Leu | Lys | Trp | Trp | Glu 330 |
| | Leu | Arg | | | | | | | | | | | | | |
| | | | | | | SEQ CERIS | | | 14: | | | | | | |
| 20 | (A) (B) | LENG | STH: E: an | - | acio | d | | | | | | | | | |
| | (C) | STR | ANDEI | ONES | 3: s: | ingle | 9 | ٠. | | | | | | | |
| | (ii) | | LECUI | LE TY | YPE: | pept | | | | | | | | | |
| 25 | (xi) |) SEÇ | QUENC | CE DE | ESCR | IPTIC | ON: S | SEQ I | ID NO |): 14 | 4: | | | | |
| | Pro 1 | Thr | Asp | Leu | Arg 5 | Phe | Thr | Asn | Ile | Gly 10 | Pro | Asp | Thr | Met | Arg 15 |
| | Val | Thr | Trp | Ala | Pro 20 | Pro | Pro | Ser | Ile | Asp 25 | Leu | Thr | Asn | Phe | Leu 30 |
| 30 | Val | Arg | Tyr | Ser | Pro 35 | Val | Lys | Asn | Glu | Glu 40 | Asp | Val | Ala | Glu | Leu 45 |
| | | | | | 50 | - | | | | 55 | | | | Leu | 60 |
| | | _ | | | 65 | | | | | 70 | | | _ | Glu | 75 |
| 35 | | | | | 80 | | _ | _ | _ | 85 | _ | | | Leu | 90 |
| | | | | _ | 95 | _ | | | _ | 100 | | | | Ser | 105 |
| | | | | | 110 | | | _ | | 115 | | | _ | Tyr | 120 |
| 40 | | | | | 125 | | | | | 130 | _ | | _ | Glu | 135 |
| | _ | | | | 140 | | | | | 145 | | | | Leu | 150 |
| 45 | | | | | 155 | | | | | 160 | | | | Gly | 165 |
| | Glu | Glu | Ser | Pro | Leu 170 | Leu | Ile | Gly | Gln | Gln 175 | Ser | Thr | Val | Ser | Asp 180 |
| | Val | Pro | Arg | Asp | Leu 185 | Glu | Val | Val | Ala | Ala 190 | Thr | Pro | Thr | Ser | Leu 195 |
| 50 | Leu | Ile | Ser | Trp | Asp 200 | Ala | Pro | Ala | Val | Thr 205 | Val | Arg | Tyr | Tyr | Arg 210 |
| | | | - | - | 215 | | _ | _ | | 220 | | | | Glu | 225 |
| | Thr | Val | Pro | Gly | Ser | Lys | Ser | Thr | Ala | Thr | Ile | Ser | Gly | Leu | Lys |

| | | | | | 230 | | | | | 235 | | | | | 240 |
|----|----------|-------------|---------------|-------|------------|-------|-----|-------|-------|------------|-----|-----|-----|-----|------------|
| | Pro | Gly | Val | Asp | | Thr | Ile | Thr | Val | | Ala | Val | Thr | Gly | Arg 255 |
| 5 | Gly | Asp | Ser | Pro | Ala 260 | Ser | Ser | Lys | Pro | Ile 265 | Ser | Ile | Asn | Tyr | Arg 270 |
| | Thr | Glu | Ile | Asp | | Pro | Ser | Met | Gly | | Tyr | Ile | Ser | Gly | |
| | Ala | Pro | Arg | Pro | | Leu | Thr | Lys | Lys | Gln 295 | Arg | Phe | Arg | His | Arg 300 |
| 10 | Asn | Arg | Lys | Gly | Tyr 305 | Arg | Ser | Gln | Arg | Gly 310 | His | Ser | Arg | Gly | Arg 315 |
| | Asn | Gln | Asn | Ser | Arg 320 | Arg | Pro | Ser | Arg | Ala 325 | Met | Trp | Leu | Ser | Leu 330 |
| | Phe | Ser | Ser | Lys | Asn 335 | Ser | Ser | Ser | Val | Pro 340 | Ala | | | | |
| 15 | | **** | | | | an. | | 70. | | | | | | | |
| | | INF | | | | | | | 15: | | | | | | |
| | (A) | LENG TYP | STH: S: ar | | acio | H | | | | | | | | | |
| 20 | (C) | STR | ANDEI | ONES | 3: s: | ingle | 9 | | : | | | | | | |
| | | TOP(:MO | | | | | ide | | | | | | | | |
| | (xi |) SE | QUEN | CE DI | ESCR. | IPTIC | ON: | SEQ : | ID NO | D: 15 | 5: | | | | |
| 25 | Pro 1 | Thr | Asp | Leu | Arg 5 | Phe | Thr | Asn | Ile | Gly 10 | Pro | Asp | Thr | Met | Arg 15 |
| | Val | Thr | Trp | Ala | Pro 20 | Pro | Pro | Ser | Ile | Asp 25 | Leu | Thr | Asn | Phe | Leu 30 |
| | Val | Arg | Tyr | Ser | Pro 35 | Val | Lys | Asn | Glu | Glu 40 | Asp | Val | Ala | Glu | Leu 45 |
| 30 | Ser | Ile | Ser | Pro | Ser 50 | Asp | Asn | Ala | Val | Val 55 | Leu | Thr | Asn | Leu | Leu 60 |
| | Pro | Gly | Thr | Glu | Tyr 65 | Val | Val | Ser | Val | Ser 70 | Ser | Val | Tyr | Glu | Gln 75 |
| | His | Glu | Ser | Thr | Pro 80 | Leu | Arg | Gly | Arg | Gln 85 | Lys | Thr | Gly | Leu | Asp 90 |
| 35 | Ser | Pro | Thr | Gly | Ile 95 | Asp | Phe | Ser | Asp | Ile 100 | Thr | Ala | Asn | Ser | Phe 105 |
| | Thr | Val | His | Trp | Ile 110 | Ala | Pro | Arg | Ala | Thr 115 | Ile | Thr | Gly | Tyr | Arg 120 |
| | Ile | Arg | His | His | | Glu | His | Phe | Ser | | Arg | Pro | Arg | Glu | |
| 40 | Arg | Val | Pro | His | Ser 140 | Arg | Asn | Ser | Ile | Thr 145 | Leu | Thr | Asn | Leu | Thr 150 |
| | Pro | Gly | Thr | Glu | Tyr 155 | Val | Val | Ser | Ile | Val 160 | Ala | Leu | Asn | Gly | Arg 165 |
| | Glu | Glu | Ser | Pro | | Leu | Ile | Gly | Gln | | Ser | Thr | Val | Ser | |
| 45 | Val | Pro | Arg | Asp | | Glu | Val | Val | Ala | | Thr | Pro | Thr | Ser | |
| | Leu | Ile | Ser | Trp | | Ala | Pro | Ala | Val | | Val | Arg | Tyr | Tyr | |
| 50 | Ile | Thr | Tyr | Gly | | Thr | Gly | Gly | Asn | | Pro | Val | Gln | Glu | |
| JU | Thr | Val | Pro | Gly | | Lys | Ser | Thr | Ala | | Ile | Ser | Gly | Leu | |
| | Pro | Gly | Val | Asp | | Thr | Ile | Thr | Val | | Ala | Val | Thr | Gly | |
| | | | | | | | | | | | | | | | |

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250
                              245
              Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                                                   265
                               260
              Thr Glu Ile Asp Lys Pro Ser Met Val Pro Gly Phe Lys Gly Asp
5
                               275
                                                                        285
                                                   280
              Met Gly Leu Lys Gly Asp Arg Gly Glu Val Gly Gln Ile Gly Pro
                               290
                                                   295
              Arg Gly Xxx Asp Gly Pro Glu Gly Pro Lys Gly Arg Ala Gly Pro
                                                   310
                               305
10
              Thr Gly Asp Pro Gly Pro Ser Gly Gln Ala Gly Glu Lys Gly Lys
                               320
                                                   325
                                                                        330
              Leu Gly Val Pro Gly Leu Pro Gly Tyr Pro Gly Arg Gln Gly Pro
                               335
                                                   340
              Lys Gly Ser Thr Gly Phe Pro Gly Phe Pro Gly Ala Asn Gly Glu
                               350
                                                   355
15
              Lys Gly Ala Arg Gly Val Ala Gly Lys Pro Gly Pro Arg Gly Gln
                               365
                                                   370
                                                                        375
              Arg Gly Pro Thr Gly Pro Arg Gly Ser Arg Gly Ala Arg Gly Pro
                               380
                                                   385
                                                                        390
              Thr Gly Lys Pro Gly Pro Lys Gly Thr Ser Gly Gly Asp Gly Pro
                               395
                                                   400
20
              Pro Gly Pro Pro Gly Glu Arg Gly Pro Gln Gly Pro Gln Gly Pro
                               410
                                                   415
              Val Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gly Arg
                               425
                                                   430
              Met Gly Cys Pro Gly His Pro Gly Gln Arg Gly
                               440
25
              (2) INFORMATION FOR SEQ ID NO: 16:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 457
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
35
                                                    10
                                                                        1.5
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                                20
                                                    25
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                               35
                                                    40
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
40
                               50
                                                    55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                                65
                                                    70
                                                                         75
              His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                               80
                                                    85
45
              Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                                                   100
                                                                        105
              Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                              110
                                                   115
              Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                              125
                                                   130
50
              Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                               140
                                                   145
              Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
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| | | | | | 155 | | | | | 1.00 | | | | , | 165 |
|----|----------|-------|----------------|-------|------------|-------|-------|--------|-------|------------|------|-----|------|-----|------------|
| | Glu | Glu | Ser | Pro | 155 Leu | Leu | Ile | Gly | Gln | 160 Gln | Ser | Thr | Val | Ser | 165 Asp |
| | 77- 7 | D | 3 | 7 | 170 | C1 | 17-1 | T7-1 | חות | 175 | mb » | Dwo | Πb ν | Sor | 180 |
| 5 | vaı | Pro | Arg | Asp | 185 | GIU | vaı | Val | Ala | 190 | TIIT | PIO | 1111 | Ser | 195 |
| | Leu | Ile | Ser | Trp | Asp 200 | Ala | Pro | Ala | Val | Thr 205 | Val | Arg | Tyr | Tyr | Arg 210 |
| | Ile | Thr | Tyr | Gly | Glu | Thr | Gly | Gly | Asn | Ser | Pro | Val | Gln | Glu | Phe |
| 10 | Thr | Val | Pro | Gly | 215 Ser | Lys | Ser | Thr | Ala | 220 Thr | Ile | Ser | Gly | Leu | 225 Lys |
| | | | | - | 230 | - | | | | 235 | | | _ | Gly | 240 |
| | | _ | | - | 245 | | | | | 250 | 4. | | | | 255 |
| | Gly | Asp | Ser | Pro | Ala 260 | Ser | Ser | Lys | Pro | Ile 265 | Ser | Ile | Asn | Tyr | Arg 270 |
| 15 | Thr | Glu | Ile | Asp | Lys | Pro | Ser | Met | Asn | Val | Ser | Pro | Pro | Arg | Arg |
| | Ala | Arg | Val | Thr | 275 Asp | Ala | Thr | Glu | Thr | 280 Thr | Ile | Thr | Ile | Ser | 285 Trp |
| | Ara | Thr | Lvs | Thr | 290 Glu | Thr | Tle | Thr | Glv | 295 Phe | Gln | Val | Asn | Ala | 300 Val |
| 20 | _ | | _ | | 305 | | | | _ | 310 | | | _ | | 315 |
| | Pro | Ala | Asn | GTĀ | G1n 320 | Thr | Pro | Ile | Gin | Arg 325 | Thr | He | Lys | Pro | 330 |
| | Val | Arg | Ser | Tyr | Thr 335 | Ile | Thr | Gly | Leu | Gln 340 | Pro | Gly | Thr | Asp | Tyr 345 |
| C_ | Lys | Ile | Tyr | Leu | Tyr | Thr | Leu | Asn | Asp | Asn | Ala | Arg | Ser | Ser | Pro |
| 25 | Val | Val | Ile | Asp | 350 Ala | Ser | Thr | Ala | Ile | 355 Asp | Ala | Pro | Ser | Asn | 360 Leu |
| | | | | _ | 365 | | | | | 370 | | | | Trp | 375 |
| | _ | | | | 380 | | | | | 385 | | | | _ | 390 |
| 30 | Pro | Pro | Arg | Ala | Arg 395 | Ile | Thr | Gly | Tyr | Ile 400 | Ile | Lys | Tyr | Glu | Lys 405 |
| | Pro | Gly | Ser | Pro | | Arg | Glu | Val | Val | | Arg | Pro | Arg | Pro | |
| | Val | Thr | Glu | Ala | 410 Thr | Ile | Thr | Gly | Leu | 415 Glu | Pro | Gly | Thr | Glu | 420 Tyr |
| 35 | Thr | Tle | Tvr | Val | 425 Tle | Ala | Len | Tivs | Asn | 430 Asn | Gln | Lvs | Ser | Glu | 435 Pro |
| 55 | | | | | 440 | | | _,_ | | 445 | | -,- | 201 | 014 | 450 |
| | Leu | TTE | Gly | Arg | Lys 455 | ьуs | Thr | | | | | | | | |
| | (2) | TNFY | ORMA | rton. | FOR | SEO | ו מד | ٠ ن | 17• | | | | | | |
| 40 | (i) | SEQ | JENCI | E CHA | | | | | - , • | | | | | | |
| | | | GTH: E: ar | | acio | i t | | | | | | | | | |
| | (C) | STR | ANDEI OLOG! | ONES | 3: s: | ingle | € | | | | | | | | |
| 45 | (ii) |) MOI | LECU1 | E T | PE: | pept | | | | | | | | | |
| | (xi) |) SE(| QUENC | CE DI | ESCR | IPTIC | ON: S | SEQ : | ID NO |): 1' | 7: | | | | |
| | _ | Thr | Asp | Leu | Arg | Phe | Thr | Asn | Ile | | Pro | Asp | Thr | Met | . = |
| | l Val | Thr | Trp | Ala | Pro | Pro | Pro | Ser | Ile | 10 Asp | Leu | Thr | Asn | Phe | 15 Leu |
| 50 | Val | Ara | ጥንንድ | Ser | 20 Pro | ۷al | Lve | Aen | Glii | 25 Glu | Aen | Val | Δl= | Glu | 30 Teu |
| | | | | | 35 | | | | | 40 | = | | | | 45 |
| | Ser | Ile | Ser | Pro | Ser | Asp | Asn | Ala | Val | Val | Leu | Thr | Asn | Leu | Leu |

| | | | | | 5 0 | | | | | 55 | | | | | 60 |
|----|-------------------|------------------------------|------------------------|----------------------|-----------------|-------|------|-------|------|------------|-----|-----|-----|-----|------------|
| | Pro | Gly | Thr | Glu | 50 Tyr 65 | Val | Val | Ser | Val | | Ser | Val | Tyr | Glu | |
| 5 | His | Glu | Ser | Thr | | Leu | Arg | Gly | Arg | | Lys | Thr | Gly | Leu | |
| | Ser | Pro | Thr | Gly | Ile 95 | Asp | Phe | Ser | Asp | Ile 100 | Thr | Ala | Asn | Ser | Phe 105 |
| | Thr | Val | His | Trp | Ile 110 | Ala | Pro | Arg | Ala | Thr 115 | Ile | Thr | Gly | Tyr | Arg 120 |
| 10 | Ile | Arg | His | His | Pro 125 | Glu | His | Phe | Ser | Gly 130 | Arg | Pro | Arg | Glu | Asp 135 |
| | Arg | Val | Pro | His | Ser 140 | Arg | Asn | Ser | Ile | Thr 145 | Leu | Thr | Asn | Leu | Thr 150 |
| 15 | Pro | Gly | Thr | Glu | Tyr 155 | Val | Val | Ser | Ile | Val 160 | Ala | Leu | Asn | Gly | Arg 165 |
| 15 | | Glu | | | 170 | | | - | | 175 | | | | | 180 |
| | | Pro | - | _ | 185 | | | | | 190 | | | | | 195 |
| 20 | | Ile | | _ | 200 | | | | | 205 | | | | | 210 |
| | | Thr | | | 215 | | | | | 220 | | | | | 225 |
| | | Val | | _ | 230 | _ | | | | 235 | | | | | 240 |
| 25 | | Gly | | | 245 | | | | | 250 | | _ | | _ | 255 |
| | | Asp | | | 260 | | | _ | _ | 265 | | | | _ | 270 |
| | | Glu - | | _ | 275 | | | | | 280 | _ | | | | 285 |
| 30 | | Arg | | | 290 | | | | | 295 | | | | | 300 |
| | | Pro | | _ | 305 | _ | | | _ | 310 | | | _ | _ | 315 |
| | | Pro | | | 320 | | _ | | | 325 | | _ | | _ | 330 |
| 35 | _ | Val | | | 335 | | | | Ā | 340 | | | - | | 345 |
| | | Thr | | | 350 | | | | Lys | Asn 355 | Asn | GIn | Lys | Ser | 360 |
| 40 | Pro | Leu | TTE | GTĀ | 365 | гля | ьуѕ | Thr | | | | | | | |
| | (i) (A) (B) | INFO SEQU LENO TYPE | JENCI GTH: E: ar | E CHI 367 mino | ARAC: | rerī: | STIC | | 18: | | | | | | |
| 45 | (D) (ii | STRA TOPO MOD SEO | LECU: | Y: 1: LE T: | inea: YPE: | pep | tide | SEQ : | ID N | o: 1 | B: | | | | |
| 50 | _ | Thr | Asp | Leu | Arg | Phe | Thr | Asn | Ile | | Pro | Asp | Thr | Met | |
| 50 | 1 Val | Thr | Trp | Ala | | Pro | Pro | Ser | Ile | | Leu | Thr | Asn | Phe | |
| | Val | Arg | Tyr | Ser | 20 Pro | Val | Lys | Asn | Glu | 25 Glu | Asp | Val | Ala | Glu | 30 Leu |
| | | | | | | | | | | | | | | | |

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35
                                                    40
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                                                    55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
5
                                                    70
                               65
              His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                               80
                                                    85
                                                                        90
              Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                               95
                                                   100
              Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
10
                                                  115 .
                              110
              Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                                                   130
                              125
              Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                              140
                                                  145
15
              Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                              155
                                                   160
              Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                              170
                                                   175
              Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                                                   190
                              185
20
              Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                              200
                                                   205
              Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                              215
                                                   220
              Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                              230
                                                   235
                                                                       240
25
              Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                              245
                                                   250
              Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                              260
                                                   265
              Thr Glu Ile Asp Lys Pro Ser Met Asn Val Ser Pro Pro Arg Arg
                              275
                                                   280
              Ala Arq Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp
                              290
                                                   295
              Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala Val
                              305
                                                   310
              Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys Pro Asp
35
                              320
                                                  325
              Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr Asp Tyr
                              335
                                                  340
                                                                       345
              Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser Pro
                              350
                                                   355
40
              Val Val Ile Asp Ala Ser Thr
                              365
              (2) INFORMATION FOR SEQ ID NO: 19:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 464
45
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:
50
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
```

Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu

| | | | | | 20 | | | | | 25 | | | | | 30 |
|-----------|-----|-----|-----|-----|------------|-----|-----|-----|------|------------|-----|-----|-----|-----|------------|
| V | /al | Arg | Tyr | Ser | Pro 35 | Val | Lys | Asn | Glu | Glu 40 | Asp | Val | Ala | Glu | Leu 45 |
| 5 S | Ser | Ile | Ser | Pro | Ser 50 | Asp | Asn | Ala | Val | Val 55 | Leu | Thr | Asn | Leu | Leu 60 |
| P | ?ro | Gly | Thr | Glu | Tyr 65 | Val | Val | Ser | Val | Ser 70 | Ser | Val | Tyr | Glu | Gln 75 |
| H | lis | Glu | Ser | Thr | Pro 80 | Leu | Arg | Gly | Arg | Gln 85 | Lys | Thr | Gly | Leu | Asp 90 |
| 10 S | Ser | Pro | Thr | Gly | Ile 95 | Asp | Phe | Ser | Asp | Ile 100 | Thr | Ala | Asn | Ser | Phe 105 |
| T | hr | Val | His | Trp | | Ala | Pro | Arg | Ala | | Ile | Thr | Gly | Tyr | |
| I | [le | Arg | His | His | | Glu | His | Phe | Ser | | Arg | Pro | Arg | Glu | |
| 15 A | ۱rg | Val | Pro | His | | Arg | Asn | ser | ·Ile | | Leu | Thr | Asn | Leu | |
| P | Pro | Gly | Thr | Glu | | Val | Val | Ser | Ile | | Ala | Leu | Asn | Gly | |
| | slu | Glu | Ser | Pro | | Leu | Ile | Gly | Gln | | Ser | Thr | Val | Ser | |
| 20 V | /al | Pro | Arg | Asp | | Glu | Val | Val | Ala | | Thr | Pro | Thr | Ser | |
| I | Leu | Ile | Ser | Trp | Asp 200 | Ala | Pro | Ala | Val | | Val | Arg | Tyr | Tyr | |
| 25 | lle | Thr | Tyr | Gly | | Thr | Gly | Gly | Asn | | Pro | Val | Gln | Glu | |
| | hr | Val | Pro | Gly | | Lys | Ser | Thr | Ala | | Ile | Ser | Gly | Leu | |
| P | Pro | Gly | Val | Asp | Tyr 245 | Thr | Ile | Thr | Val | | Ala | Val | Thr | Gly | Arg 255 |
| <i>30</i> | Sly | Asp | Ser | Pro | | Ser | Ser | Lys | Pro | | Ser | Ile | Asn | Tyr | |
| T | hr | Glu | Ile | Asp | Lys 275 | Pro | Ser | Met | Gly | | Arg | Gly | Leu | Lys | |
| T | hr | Lys | Gly | Glu | Lys 290 | Gly | Glu | Asp | Gly | Phe 295 | Pro | Gly | Phe | Lys | _ |
| 35 A | Asp | Met | Gly | Ile | Lys 305 | Gly | Asp | Arg | Gly | | Ile | Gly | Pro | Pro | |
| P | Pro | Arg | Gly | Glu | Asp 320 | Gly | Pro | Glu | Gly | Pro 325 | Lys | Gly | Arg | Gly | |
| P | ?ro | Asn | Gly | Asp | | Gly | Pro | Leu | Gly | | Pro | Gly | Glu | Lys | |
| 40 1. | Jys | Leu | Gly | Val | | Gly | Leu | Pro | Gly | | Pro | Gly | Arg | Gln | |
| P | Pro | Lys | Gly | Ser | | Gly | Phe | Pro | Gly | | Pro | Gly | Ala | Asn | Gly 375 |
| | Glu | Lys | Gly | Gly | | Gly | Thr | Pro | Gly | | Pro | Gly | Pro | Arg | Gly 390 |
| 45 G | 3ln | Arg | Gly | Pro | | Gly | Pro | Arg | Gly | | Arg | Gly | Pro | Arg | |
| I | [le | Thr | Gly | Lys | | Gly | Pro | Lys | Gly | | Ser | Gly | Gly | Asp | |
| 50 | Pro | Ala | Gly | Pro | | Gly | Glu | Arg | Gly | | Asn | Gly | Pro | Gln | |
| | Pro | Thr | Gly | Phe | | Gly | Pro | Lys | Gly | | Pro | Gly | Pro | Pro | |
| L | Ъуs | Asp | Gly | Leu | | Gly | His | Pro | Gly | | Arg | Gly | Glu | Thr | 400 |

455 460

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(2) INFORMATION FOR SEQ ID NO: 20:(i) SEQUENCE CHARACTERISTICS:
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(A) LENGTH: 432

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu .185 Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg Thr Glu Ile Asp Lys Pro Ser Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn Arg Tyr Leu

| | | | | | 350 | | | | | 355 | | | | | 360 |
|----|---|---------------------------|---|---|--|------------------------|-------|-------|---------|-------------------|-----|-----|-----|-----|------------|
| | | | _ | | 365 | | | | | 370 | | | | Val | 375 |
| 5 | Asp | Glu | Cys | Phe | Phe 380 | Phe | Glu | Arg | Leu | Glu 385 | Ser | Asn | Asn | Tyr | Asn 390 |
| | Thr | Tyr | Arg | Ser | Arg 395 | Lys | Tyr | Thr | Ser | Trp 400 | Tyr | Val | Ala | Leu | Lys 405 |
| | Arg | Thr | Gly | Gln | Tyr 410 | Lys | Leu | Gly | Ser | Lys 415 | Thr | Gly | Pro | Gly | Gln 420 |
| 10 | Lys | Ala | Ile | Leu | Phe 425 | Leu | Pro | Met | Ser | Ala 430 | Lys | Ser | | | |
| 15 | (i) (A) (B) (C) (D) (ii) | SEQUENCE TYPE STRATE TOPO | JENCI GTH: E: ar ANDEI DLOGY LECUI | E CHA 574 mino ONESS (: 1: LE T) | acio acio S: s: inea: (PE: | TERIS ingle pept | STIC: | | 21: | n: 2 ⁻ | ·. | | | | |
| 20 | (22.1) | | 201111 | יט טי | 1001(1 | | | , VDC | 10 | · | • | | | | |
| 20 | Pro 1 | Thr | Asp | Leu | Arg 5 | Phe | Thr | Asn | Ile | Gly 10 | Pro | Asp | Thr | Met | Arg 15 |
| | Val | Thr | Trp | Ala | Pro 20 | Pro | Pro | Ser | Ile | Asp 25 | Leu | Thr | Asn | Phe | Leu 30 |
| 25 | Val | Arg | Tyr | Ser | Pro 35 | Val | Lys | Asn | Glu | Glu 40 | Asp | Val | Ala | Glu | Leu 45 |
| | Ser | Ile | Ser | Pro | Ser 50 | Asp | Asn | Ala | Val | Val 55 | Leu | Thr | Asn | Leu | Leu 60 |
| | Pro | Gly | Thr | Glu | Tyr 65 | Val | Val | Ser | Val | Ser 70 | Ser | Val | Tyr | Glu | Gln 75 |
| 30 | His | Glu | Ser | Thr | Pro 80 | Leu | Arg | Gly | Arg | Gln 85 | Lys | Thr | Gly | Leu | Asp 90 |
| | Ser | Pro | Thr | Gly | Ile 95 | Asp | Phe | Ser | Asp | Ile 100 | Thr | Ala | Asn | Ser | Phe 105 |
| | Thr | Val | His | Trp | Ile 110 | Ala | Pro | Arg | Ala | Thr 115 | Ile | Thr | Gly | Tyr | Arg 120 |
| 35 | Ile | Arg | His | His | Pro 125 | Glu | His | Phe | Ser | Gly 130 | Arg | Pro | Arg | Glu | Asp 135 |
| | Arg | Val | Pro | His | Ser 140 | Arg | Asn | Ser | Ile | Thr 145 | Leu | Thr | Asn | Leu | Thr 150 |
| | Pro | Gly | Thr | Glu | Tyr 155 | Val | ۷al | ser | Ile | Val 160 | Ala | Leu | Asn | Gly | Arg 165 |
| 40 | Glu | Glu | Ser | Pro | Leu 170 | Leu | Ile | Gly | Gln | Gln 175 | Ser | Thr | Val | Ser | Asp 180 |
| | Val | Pro | Arg | Asp | Leu 185 | Glu | Val | Val | Ala | Ala 190 | Thr | Pro | Thr | Ser | Leu 195 |
| | Leu | Ile | Ser | Trp | Asp 200 | Ala | Pro | Ala | Val | Thr 205 | Val | Arg | Tyr | Tyr | Arg 210 |
| 45 | Ile | Thr | Tyr | Gly | Glu 215 | Thr | Gly | Gly | Asn | Ser 220 | Pro | Val | Gln | Glu | Phe 225 |
| | Thr | Val | Pro | Gly | | Lys | Ser | Thr | Ala | | Ile | Ser | Gly | Leu | |
| 50 | Pro | Gly | Val | Asp | | Thr | Ile | Thr | Val | | Ala | Val | Thr | Gly | |
| | Gly | Asp | Ser | Pro | | Ser | Ser | Lys | Pro | | Ser | Ile | Asn | Tyr | |
| | Thr | Glu | Ile | Asp | | Pro | Ser | Met | Ala | | Pro | Ala | Pro | Thr | |

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275
                                                280
                                                                     285
          Leu Lys Phe Thr Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp
                           290
                                                295
                                                                      300
          Thr Pro Pro Asn Val Gln Leu Thr Gly Tyr Arg Val Arg Val Thr
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                                                310
                                                                      315
                           305
          Pro Lys Glu Lys Thr Gly Pro Met Lys Glu
                                                    Ile Asn Leu Ala Pro
                           320
                                                325
                                                                     330
          Asp Ser Ser Ser Val Val Val Ser Gly Leu Met Val Ala Thr Lys
                           335
                                                340
                                                                     345
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          Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr Ser Arg
                           350
                                                355
                                                                     360
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                                                370
                                                                     375
          Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile
15
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                                                385
                                                                      390
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                           395
                                                                      405
                                                400
          Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys
                           410
                                                415
                                                                      420
20
          Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr
                           425
                                                430
                                                                      435
          Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser
                           440
                                                445
                                                                     450
          Ser Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser
25
                           455
                                                460
                                                                      465
          Asn Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser
                           470
                                                475
                                                                     480
          Trp Gln Pro Pro Arg Ala Arg Ile Thr Gly
                                                    Tyr Ile Ile Lys
                                                                     Tyr
                           485
                                                490
                                                                      495
30
          Glu Lys Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg
                           500
                                                505
                                                                     510
          Pro Gly Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr
                           515
                                                520
                                                                     525
          Glu Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser
35
                           530
                                                535
                                                                     540
          Glu Pro Leu Ile Gly Arg Lys Lys Thr Asp Glu Leu Pro Gln Leu
                           545
                                                550
          Val Thr Leu Pro His Pro Asn Leu His Gly Pro Glu Ile Leu Asp
                           560
40
          Val Pro Ser Thr
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Claims

- In a method for production of transfected cells by transferring a foreign gene into target cells using a perforation method, said method for production of cells transfected with a foreign gene which comprises a step of, after injection of a foreign gene into target cells using a perforation method, culturing the cells in the presence of a cell-adhering active substance.
- The method for production of transfected cells according to claim 1, the culturing step is a step of culturing using a culture wear covered with a cell-adhering active substance.
- 3. The method for production of transfected cells according to claim 1, wherein the cell-adhering active substance is a cell-adhering active polypeptide or a functional equivalent of said polypeptide.
- 4. The method for production of transfected cells according to claim 3, wherein the cell-adhering active polypeptide is

a cell-adhering and/or cell-spreading active polypeptide.

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- 5. The method for production of transfected cells according to claim 3, wherein the cell-adhering and/or cell-spreading active polypeptide is a polypeptide containing the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2.
- 6. The method for production of transfected cells according to claim 3, wherein the cell-adhering active polypeptide is selected from polypeptides represented by SEQ ID: Nos. 3, 4 and 5.
- 7. The method for production of transfected cells according to claim 1, wherein the cell-adhering active substance is poly-N-p-vinylbenzyl-D-lactoneamide.
 - 8. The method for production of transfected cells according to claim 1, wherein the target cells are selected from hematopoiesis stem cell, peripheral blood stem cell, umbilical blood cell, ES cell, lymphocyte and cancer cell.
 - 9. The method for production of transfected cells according to claim 1, wherein the foreign gene is nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes).
- 10. The method for production of transfected cells according to claim 1, wherein the foreign gene is nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes) and the nucleic acid is incorporated into the vector.
- 25 11. The method for production of transfected cells according to claim 1, wherein the vector is a vector selected from retrovirus vector, adenovirus vector, vacciniavirus vector and herpesvirus vector.
 - 12. The method for production of transfected cells according to claim 1, the perforation method is selected from an electroporation method, a microinjection method and a particle gun method.
 - 13. Transfected cells produced by a method for production of transfected cells according to claim 1.
 - **14.** A kit for production of transfected cells with a foreign gene which is used in a method for production of transfected cells according to claim 1, said kit comprises containing a cell-adhering active substance.

Fig. 1

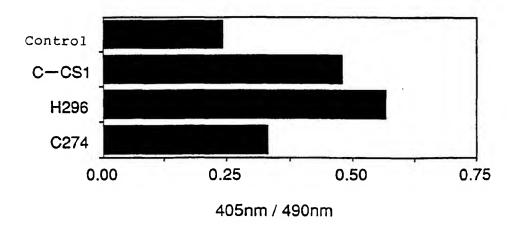
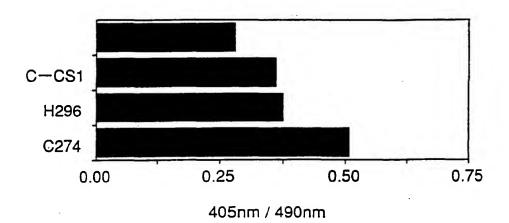


Fig. 2



INTERNATIONAL SEARCH REPORT International application No. PCT/JP95/02425 A. CLASSIFICATION OF SUBJECT MATTER Int. Cl⁶ Cl2N15/87, Cl2N5/10, C07K14/78 According to International Patent Classification (IPC) or to both national classification and IPC FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int. Cl6 C12N15/87, C12N5/10, C07K14/78 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPI, WPI/L, BIOSIS PREVIEWS CAS ONLINE C. DOCUMENTS CONSIDERED TO BE RELEVANT Category* Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. JP, 4-063597, A (W.R. Grace & Co.), 1 - 14February 28, 1992 (28. 02. 92) & EP, 463508, A & CA, 2044307, A 1 - 14JP, 6-090771, A (Shiseido Co., Ltd.), Α April 5, 1994 (05. 04. 94) (Family: none) See patent family annex. Further documents are listed in the continuation of Box C. later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance

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